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The Yale Journal of Biology & Medicine

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John R. Paul is professor of preventive medicine at Yale.

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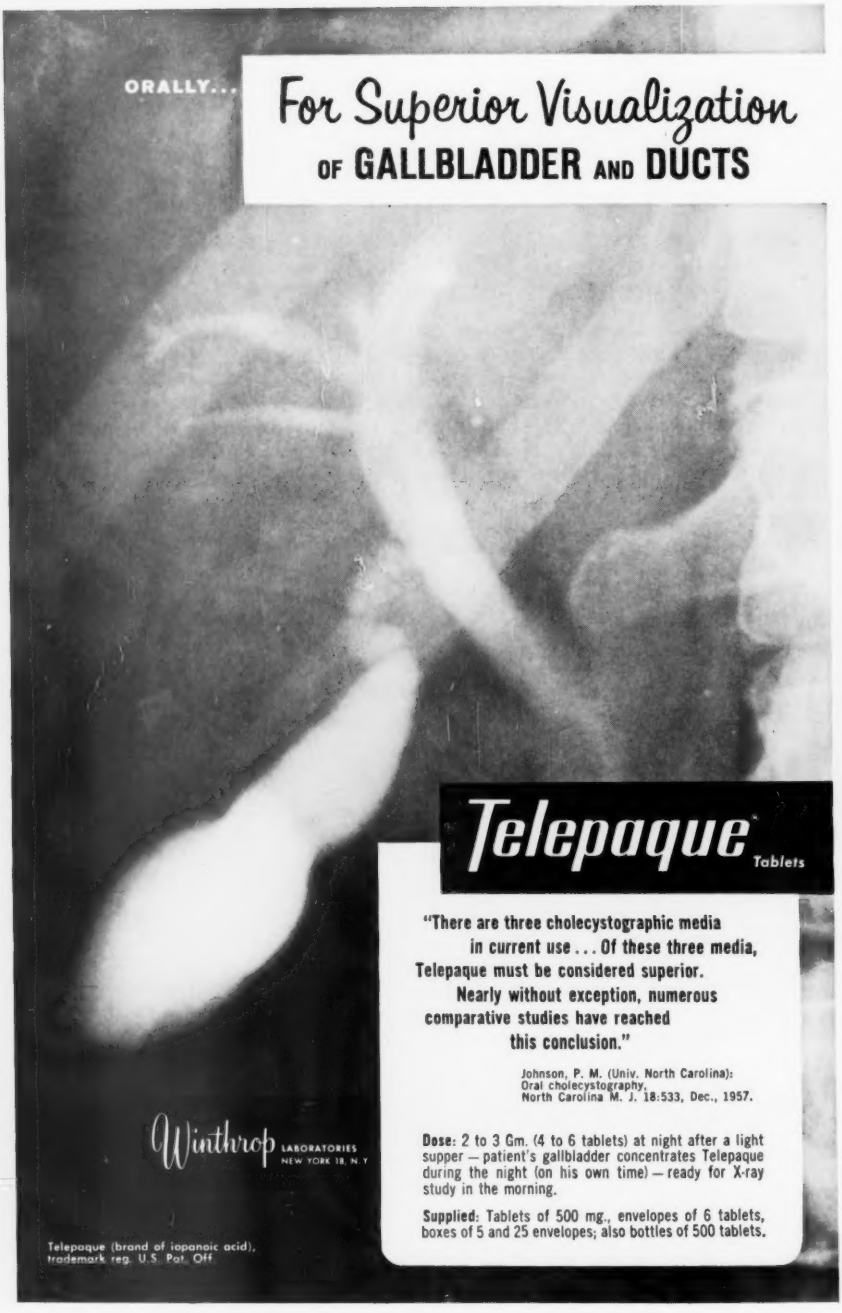

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AN ELECTRONIC RESUSCITATOR FOR POSSIBLE USE IN ASPHYXIA NEONATORUM†

Many methods have been proposed for resuscitation of the newborn but there is as yet no single widely accepted approach to this commonly recurring problem.

It is the purpose of this paper to review briefly the electronic methods of resuscitation and to describe animal experiments with a simple electronic apparatus which may be of value for the human newborn.

In 1948 Sarnoff *et al.*[7] reported animal experiments with a phrenic nerve stimulator. Even after complete cessation of spontaneous respiration following injection of one per cent procaine hydrochloride into the posterior fossa of the brain, the animal could be kept alive with the stimulator.

In human experiments using wire stimulating electrodes wrapped around the phrenic nerve, Whittenberger, Sarnoff, *et al.*[9] showed that significantly greater than normal minute volumes were obtained even though sub-maximal stimulation was used. Some patients reported shoulder pain but no permanent nerve damage was found.

Cross and Roberts[3] in 1950 reported 25 successes in 29 resuscitation attempts using a phrenic nerve stimulator on infants suffering from asphyxia neonatorum. The four failures occurred in a group of nine infants classified by them as severely ill.

Knodt[5,6] in 1951 described his experience with an electro-lung designed in 1950 by Hofmann. The electrodes were placed in such a manner as to stimulate alternately the diaphragm (inspiration), and the abdominal muscles (expiration). For inspiration, a belt consisting of two metal electrode strips separated from each other by two strips of insulating material was placed around the thorax at the level of the diaphragm, passing in front of the xyphoid process, so that one electrode was over the anterior chest and one on the back. Stimulation of the anterior thorax caused contraction of

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the diaphragm. Expiration was obtained by similarly placing a set of electrodes around the lower abdomen. Knodt suggests that the electro-lung might work through direct stimulation of the diaphragm, as contrasted to Sarnoff's method of direct stimulation of the phrenic nerve. However, the actual site of action has not been determined.

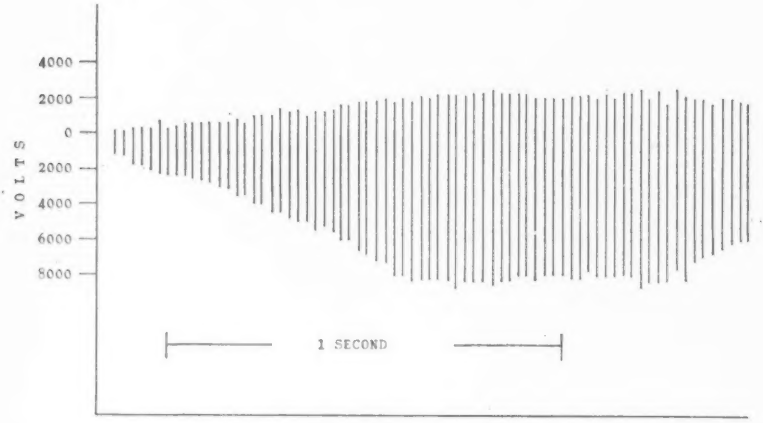

FIG. 1. Modulated wave form showing the gradual increase in pulse amplitude. Tracing made from the face of an oscilloscope.

Alliaume and Charpentier[1] also used the electro-lung and reported two cases of diaphragmatic paralysis in infants successfully treated with short periods of electro-respiration.

In spite of the efficiency of the electro-phrenic stimulator and the electro-lung, neither resuscitator has met with wide acceptance. This is probably due to the difficulty in locating the phrenic nerve with the Sarnoff stimulator and the rather complicated electrode placement needed with the electro-lung. The use of stimulating currents in the milliampere range and relatively low stimulating voltages make it important to reduce the skin resistance as much as possible.

The electronic resuscitator to be described, in contradistinction to the above-mentioned stimulators, employs currents in the microampere range

and relatively high voltages. This type of stimulus is less painful and is not appreciably altered by high contact resistance. The net effect is a resuscitator that is simple and rapid to operate.

APPARATUS

The stimulator used in these experiments was built by Batrow Laboratories, Inc., of Branford, Connecticut.

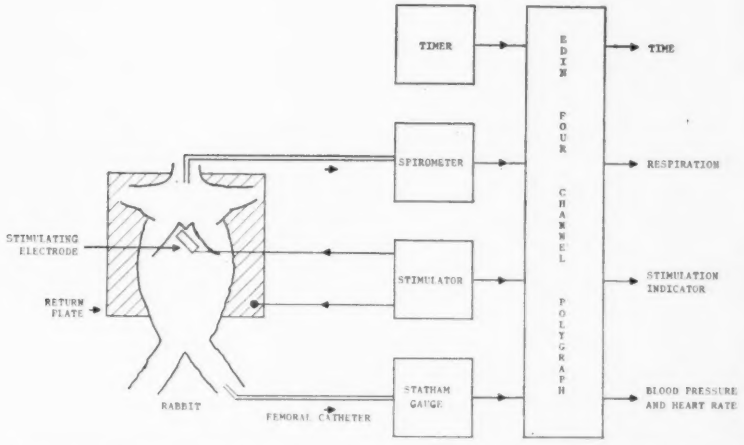

FIG. 2. Block diagram of experimental apparatus. The stimulating electrode is below the xyphoid process and the indifferent electrode (return plate) is under the rabbit.

Stimulation is provided by a series of condenser discharges fired through a thyratron tube at 45 pulses per second. The amplitude of the stimulus can be adjusted within certain limits. With a load of 100 megohms it is about 8,000 volts with a current in the range of 25 microamperes. The stimulating pulse is 300 microseconds in duration, and the amplitude of individual spikes varies in the manner shown in Figure 1.

With stimulation the diaphragm contracts and causes inspiration. Expiration is passive and takes place between periods of stimulation. To achieve inspiration in the rabbit about two-fifths of maximum intensity is required.

An indifferent electrode (12″ x 12″ metal plate) large enough to cover the back at the level of the diaphragm and a small, round, metal-tipped stimulating electrode are used. The animal is placed supine on the indifferent electrode and the stimulating electrode placed high in the epigastric region just lateral to the xyphoid process. A moist towel is put between the skin and the indifferent electrode, and a small piece of moist gauze is wrapped around the metal tip of the stimulating electrode (Fig. 2).

With diaphragmatic contraction a certain amount of pectoral and shoulder muscle contraction is also noted. A smaller indifferent electrode (2″ x 5″) was used in an

attempt to reduce the accessory muscle movement but this was of little help and had the disadvantage of requiring accurate placement of the stimulating electrode.

The respiratory rate can be varied between 12 and 50 inspirations per minute. The length of the inspiratory phase is somewhat dependent upon the respiratory rate and varies from about one-third of the total cycle at 12 respirations per minute to about one-fifth of the total cycle at higher rates.

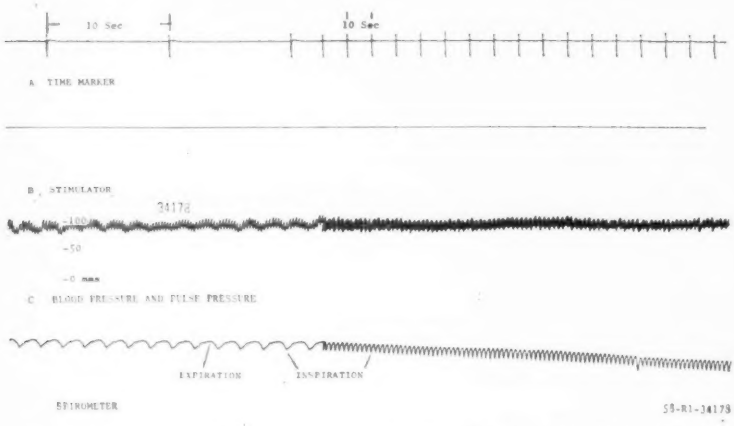

FIG. 3. Normal record. The stimulator is off. Rabbit anesthetized with pentobarbital sodium 60 mgm/5 lb.

EXPERIMENTAL PLAN

In evaluation of the apparatus experiments have been designed to provide the following information:

1. The efficiency of the stimulator in initiating and maintaining respiration in rabbits made totally apneic with barbiturates.
2. The mechanism of stimulator action:
   a. To determine if the stimulator action is dependent upon an intact respiratory center, one per cent procaine hydrochloride was injected into the contents of the posterior fossa of the skull.
   b. To determine if the site of action is the peripheral motor nerves (especially the phrenic nerves), both phrenic nerves were severed.
   c. To determine if the muscles of the diaphragm were being stimulated directly, the myoneural junction was blocked with succinylcholine chloride.
3. The possible injurious effects of the stimulator:
   a. Effects of prolonged stimulation
   b. Spontaneous inhibition of respiration
   c. Cardiac effects
   d. Local injury
   e. Miscellaneous effects.

EXPERIMENTS

Efficacy of the machine

Method. Fourteen rabbits, weighing between four and one-half and seven pounds, were anesthetized with pentobarbital sodium (60 mgm/5 lbs). A tracheotomy was performed and a tracheotomy tube connected to a Krogh spirometer. The system was filled with oxygen. Edison Baralyme granules were used to absorb carbon dioxide. Movement of the spirometer was meas-

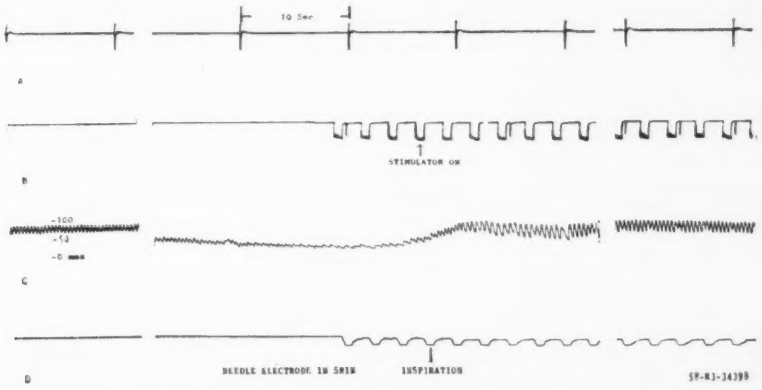

FIG. 4A. Effect of stimulator on apneic rabbit (thiopental sodium 6.4 mgm/lb.). After 70 seconds of apnea B.P. falls from 85/65 to 35/30, six inspirations bring B.P. back to 85/65. (The above record has been cut into three sections. There is a lapse of 58 seconds between sections 1 and 2 and 10 seconds between 2 and 3.)

ured by the change in potential across a variable resistor attached to the moving portion of the spirometer and recorded on one channel of an Edin four channel polygraph.

The blood pressure, pulse pressure, and heart rate were monitored by inserting a small polyethylene catheter, containing a 5 per cent solution of sodium citrate, into the femoral artery. The catheter was connected by a water column to a Statham pressure gauge whose output was recorded on a second channel of the polygraph.

Another polygraph channel was used for ten-second time intervals while the remaining channel recorded the times when the stimulator was on (Figs. 2, 3).

The blood pressure and heart rate were not recorded during all experiments. When the blood pressure was not recorded the tracings were made

by an ink pen writer attached to the moving portion of the spirometer and recorded on a continuously recording kymograph.

Forty-five minutes were required to insert the tracheotomy tube and femoral catheter. At the end of these procedures the rabbits were well anesthetized but not apneic. They were next given intravenous thiopental sodium (5-15 mgm/lb.) until apneic. Resuscitation was then attempted with the stimulator.

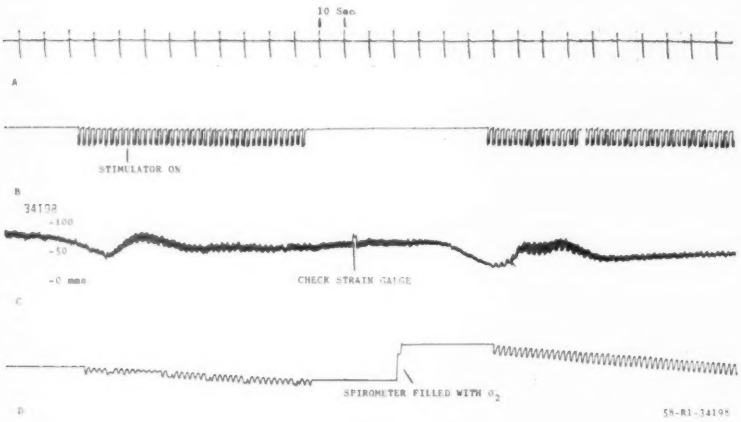

FIG. 4B. (Condensed record showing effect of stimulation on apneic rabbit (thiopental sodium 5.5 mgm/lb.). After 70 seconds of apnea B.P. drops from 80/70 to 50/30. B.P. rises after six inspirations. The second drop in B.P. comes after 60 seconds more of apnea. B.P. drops to 30/25 and rises again when respiration begins. Note rise in pulse pressure with respiration.

Results. Of the 14 rabbits studied in this manner, 13 showed signs of recovery after a few seconds of stimulation. One rabbit died immediately after a large dose (13 mgm/lb.) of intravenous thiopental sodium. One rabbit was maintained for 25 minutes with the stimulator and then suddenly died because the stimulator was no longer effective. This was attributed to fatigue of the diaphragm (see under Toxicity, infra). Twelve rabbits went on to recover completely after 15-20 minutes of stimulation.

Discussion. Figures 4A and 4B show the effect of the stimulator on two rabbits made apneic until a fall in blood pressure was noted. After a few inspirations the pulse pressure and blood pressure rapidly recovered. The results illustrated by these tracings were obtained repeatedly and indicate

that the stimulator is capable of initiating and maintaining respiration in rabbits which have received otherwise lethal doses of barbiturates.

Although respiratory volumes were not quantitatively measured, it seems that this apparatus is capable of producing minute volumes equal to, or greater than normal. This was determined by comparing the oxygen consumption of the lightly anesthetized rabbit with the oxygen consumption of

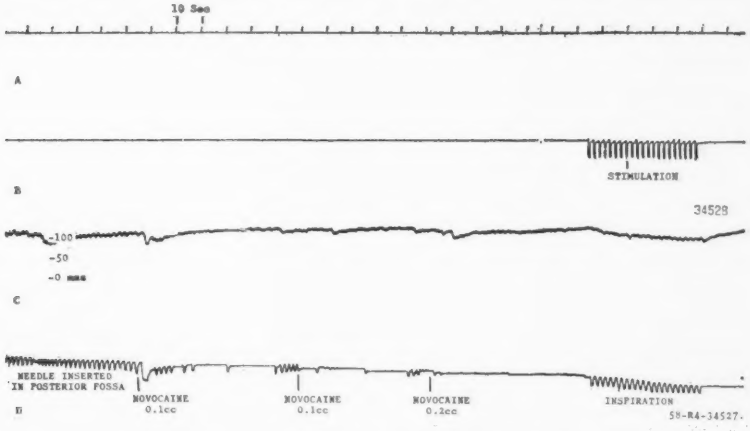

FIG. 5A. Procaine HC1 1% (0.5 cc) injected into the contents of the posterior fossa of the skull. With the respiratory center paralyzed the stimulator is able to produce inspiration.

the apneic rabbit being respirated by the Batrow stimulator. The oxygen consumption can be estimated from the slope of the respiratory tracing in much the same manner that oxygen consumption is calculated from the Benedict-Roth spirometer in determining the basal metabolic rate.

Mechanism of stimulator action

Three sets of experiments were done. In all the experiments the rabbits were prepared as described above, then the following procedures carried out.

Posterior fossa injection. In the first set of experiments one per cent procaine hydrochloride (0.5-1 cc) was injected into the contents of the posterior fossa of the skull of three rabbits. With the injection of procaine, respiration ceased immediately and the animal became completely unresponsive. The stimulator was then applied.

The respiratory arrest produced by the procaine was immediately overcome by the stimulator. Figures 5A and 5B demonstrate the reinstitution and maintenance of adequate respiration.

Phrenic nerve division. The second set of experiments was carried out on four rabbits and involved bilateral dissection and severance of the phrenic nerves in the base of the neck. At the end of the experiment the course of the phrenic nerves was traced to be sure they had been completely divided.

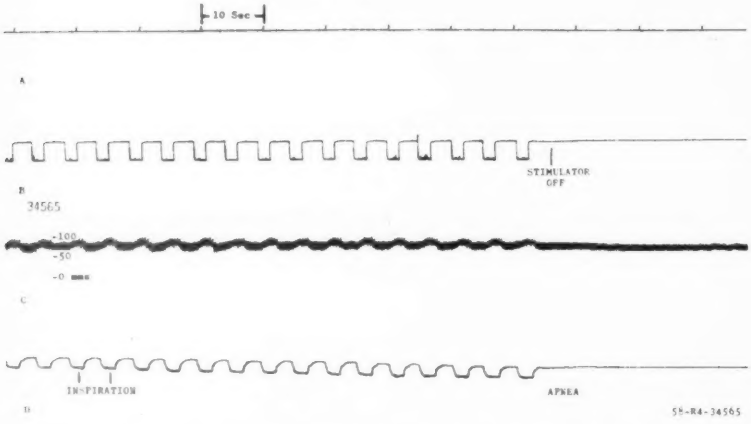

FIG. 5B. Same experiment as shown in Figure 5A but 41 minutes later demonstrating the ability of stimulator to produce inspiration after an extended period.

Figures 6A and 6B show the stimulator producing good inspiration after both phrenic nerves have been severed. In Figure 6A, with both nerves cut, the rabbit was still able to move very small amounts of oxygen with accessory muscles. Figure 6B shows the same rabbit 45 minutes later. The respiration due to the accessory muscles has completely ceased. However, the stimulator is still able to initiate and maintain respiration.

Actual diaphragmatic movement was confirmed by direct observation in two cases by opening the abdominal cavity. With the phrenic nerves severed, no contraction could be seen; with stimulation, the diaphragm immediately contracted.

Succinylcholine chloride injection. For the final set of experiments succinylcholine chloride (0.5-1 mgm/rabbit) was injected intravenously into three rabbits. Respiration ceased immediately and the animals became completely flaccid. The stimulator was then applied.

Succinylcholine completely blocked the action of the stimulator (Fig. 7). Even at high intensities the stimulator was unable to cause any inspiratory movements. As the effect of the succinylcholine wore off, the stimulator again became effective.

Discussion. Since the phrenic nerve is probably the only major motor nerve to the diaphragm,[2] it is evident from these experiments that the stimulator produces contraction of the diaphragm through stimulation of the

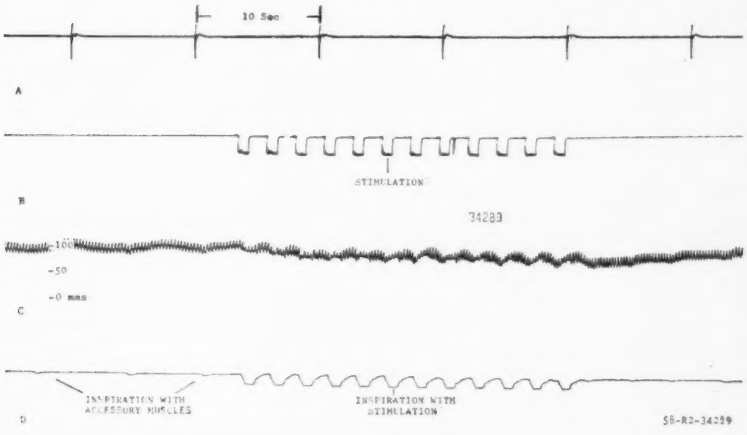

FIG. 6A. With both phrenic nerves cut, the stimulator is able still to produce inspiration. Note the small respiratory movements produced by the intercostal and accessory muscles.

phrenic nerves, either as they traverse the thoracic cavity or at their terminal arborizations on the diaphragm.

This is particularly important if this stimulator is to be used in asphyxia neonatorum. In the newborn with respiratory distress, the higher centers of respiration are often depressed, either by anesthetics or by anoxia. Since this stimulator does not depend on an intact respiratory center, it possesses a significant theoretical advantage for resuscitation under these circumstances.

From the experiments with succinylcholine it can be stated also that the myoneural junction must be intact if respiration is to be produced with this stimulator.

Injurious effects

Under the heading of injurious effects have been included not only the mechanisms by which the infant might suffer harm directly from the machine, but certain limitations of the stimulator that might be a source of danger if not recognized.

Fatigue of the diaphragm. A major limitation of this stimulator was encountered when first used on rabbits. In the early experiments pento-

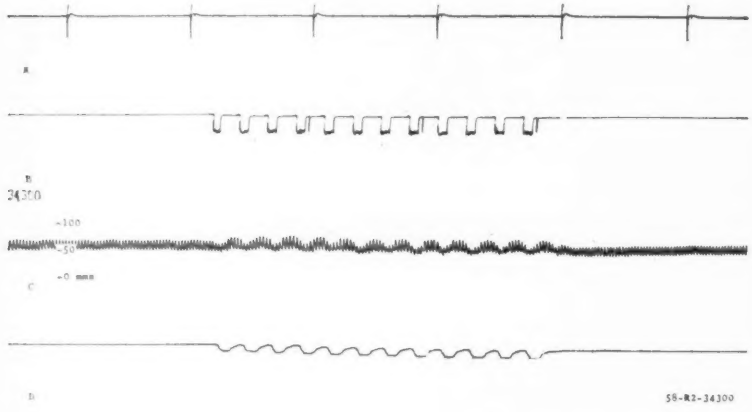

FIG. 6B. Stimulator producing respirations 34 minutes after both phrenic nerves were cut. Intercostal and accessory muscle respiratory movements have ceased.

barbital sodium was the only anesthetic used. One dose was given to anesthetize the rabbit for insertion of the tracheotomy tube and a second dose given to produce apnea. With pentobarbital the period of apnea lasted over an hour. In these apneic rabbits the stimulator was effective for 30-45 minutes and then suddenly, within two or three minutes, became ineffective and the rabbits died of apnea. This was also noted in the one rabbit (supra) that received thiopental sodium and died after 45 minutes because the stimulator was no longer effective.

Initially, this phenomenon was attributed to vascular collapse, but it soon became apparent that the lack of response was due to diaphragmatic fatigue. Careful observation showed that after 45 minutes the respirations gradually diminished until they suddenly disappeared. To check this, the stimulator was shut off and the animal given 10 to 20 positive pressure respirations. Following positive pressure breathing the diaphragm recovered and again

responded to stimulation. The response was short-lived, however, and after a few more minutes of stimulation it again became unresponsive. Positive pressure breathing again made it responsive for a brief period.

Figure 8 shows an instance of diaphragmatic fatigue and the response after five breaths with positive pressure breathing.

Spontaneous inhibition of respiration. Sarnoff, *et al.*,[8] noted spontaneous inhibition of the animals' respiration by a phrenic nerve stimulator. How-

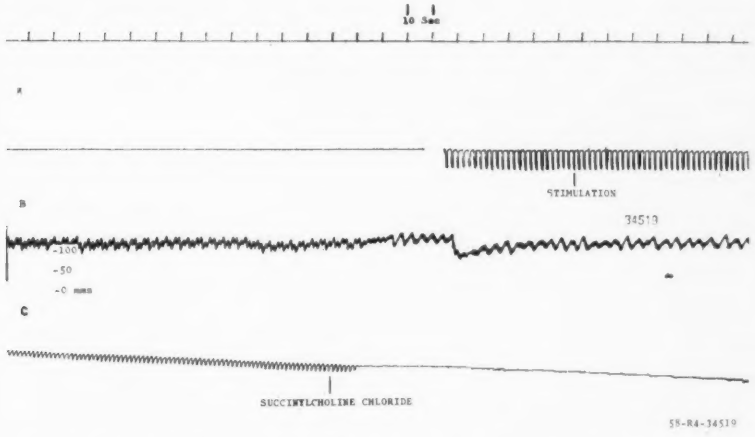

FIG. 7. Succinylcholine chloride (0.5 mgm) injected intravenously produces cessation of respiration within seconds. Stimulation at maximum intensity fails to induce any respiratory movements.

ever, Cross and Roberts[8] did not report a similar finding when using a phrenic nerve stimulator in asphyxia neonatorum.

Spontaneous inhibition was not noted in any of the rabbits in this study with the Batrow stimulator. However, the stimulator was able to interrupt the rabbit's respiration by forcing it to inspire each time the stimulus occurred, even though the rabbit continued to breathe at its normal rate between pulses from the stimulator. When the stimulus occurred the rabbit was unable to overcome the force exerted and had no choice but to inspire, and hold inspiration as long as the stimulation lasted. The intensity had to be increased in order to do this in a lightly anesthetized rabbit, but it could be done without difficulty (Fig. 9).

Cardiac effects. One of the major problems associated with an electronic stimulus is the possibility of producing cardiac arrhythmias. This is par-

ticularly true of a heart affected by prolonged anoxia. Because of the high voltage of the stimulating pulses it was not possible to monitor the heart with an electrocardiograph. Pulse rate and blood pressure were therefore used to check possible cardiovascular effects.

With the usual intensity and frequency used for resuscitation of the rabbits, no arrhythmias or abnormalities in the heart rate could be detected. The only change noted was a rise in the blood pressure when the stimulator was on. The rabbit's normal respiration usually produced a slight drop in

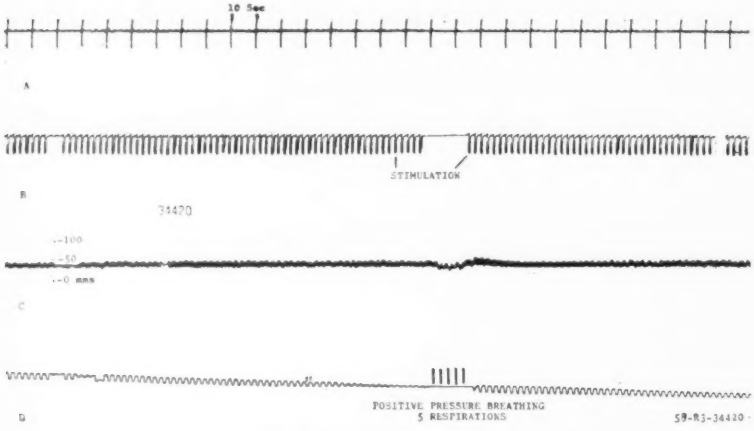

FIG. 8. Fatigue of the diaphragm in an apneic rabbit (thiopental sodium 9 mgm/lb.). The diaphragm became unresponsive after 20 minutes of stimulation. After five respirations with positive pressure breathing the diaphragm responded once again to stimulation.

blood pressure with inspiration. The exact mechanism is not clear but the rise in blood pressure produced by stimulation is probably a mechanical effect. The possible mechanism is as follows: during stimulation the thoracic cavity is expanded for a longer period than with normal inspiration. The volume of the pulmonary vascular bed is increased causing a decrease in the blood flow to the left heart and, as a result, decreased cardiac output for a few seconds. This decreased output may stimulate the carotid sinus and a compensatory rise in blood pressure is reflexly produced.

Because of these cardiovascular effects several experiments were done to see what effect extreme degrees of stimulation might have on the blood pressure and heart rate. In Figure 10A the stimulating electrode was placed over the xyphoid process and the stimulating intensity turned to maximum

(more than twice the intensity needed to produce contraction of the diaphragm). A rise in blood pressure was noted and there was also a transient slowing of the heart at the end of each stimulation. This arrhythmia may be due to a mechanical factor based on a blood pressure rise with reflex cardiac slowing; or it may be the result of direct stimulation of the vagus nerve (infra). Slowing of the heart rate is not always present, even at maximum intensity.

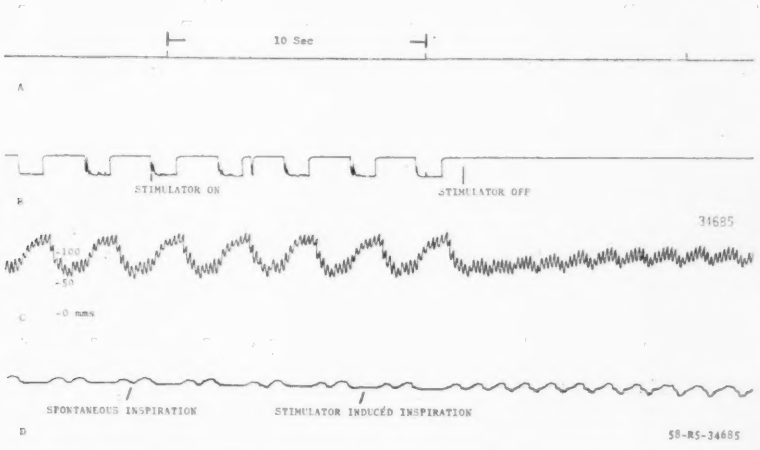

FIG. 9. Stimulator induced inspiration in non-apneic rabbit anesthetized with pentobarbital sodium 17 mgm/lb. When the stimulator is firing, the rabbit is held in inspiration. Between stimuli the rabbit carries on normal respiration.

Figure 10B shows a record taken with the stimulating electrode *over the heart,* at maximum intensity. With stimulation, the rise in blood pressure is seen again but slowing of the heart is not particularly noticeable.

Figures 11A and 11B were made by taking a needle electrode and pushing it through the chest wall until it was on the heart. The first of these records (Fig. 11A) shows the effect on the heart rate and blood pressure when the usual stimulating intensity is used.

In Figure 11B the stimulating intensity is maximum. This type of graph showing slowing of the heart rate with a drop in blood pressure could be produced regularly at high intensities with the stimulating needle directly on the heart. The exact mechanism is not known, but two possibilities will be considered:

1. The findings are the result of direct stimulation of the cardiac musculature giving a sustained, tonic type of systolic contraction with a subsequent fall in blood pressure.

2. The effect is due to stimulation of the vagus nerve. Stimulation of the vagus usually slows the whole heart; produces varying degrees of heart block or arrests the ventricles altogether; diminishes the force of contraction and shortens the duration of systole.[4] In the cat, dog or rabbit, stimulation of the vagus with a faradic current produces a change similar to those noted

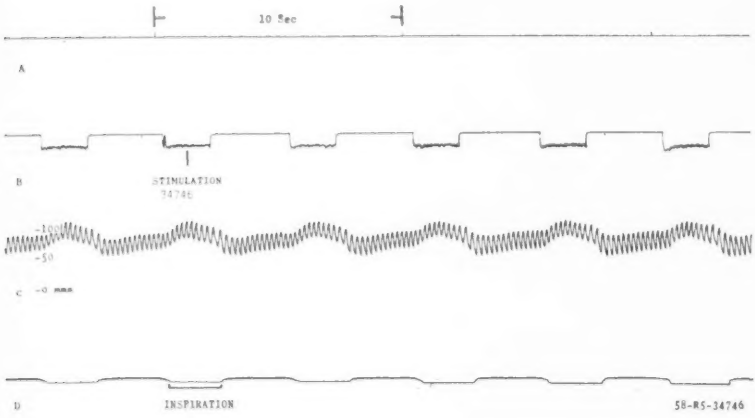

FIG. 10A. Stimulation at maximum intensity over the sternum (normal functional position for stimulating electrode). Note the slowing of the heart rate at the end of each stimulation (? vagal effect).

in Figure 11B. It is probable that both mechanisms play a part in producing the fall in blood pressure.

About ten seconds after the stimulation was stopped (Fig. 11B), the rabbit developed a series of dropped beats or ventricular asystoles which lasted 20 seconds and then ceased spontaneously. This was seen on two other occasions when abnormally high intensities were used. It is not known whether these dropped beats were due to persistent vagal effect, to premature auricular beats, or to some other cause. Dropped beats were not seen with normal stimulating intensity.

Local injury. No damage to the skin was noted as long as the stimulating electrode was held on the skin. If it were held a few millimeters from the

skin, a spark would jump from the electrode to the skin and occasionally produce a superficial burn.

Nerve damage. Fender[4] showed that continuous stimulation of the splanchnic nerves in dogs eight hours a day, six days a week, for five and one-half months with a faradic current produced no damage to the nerves.

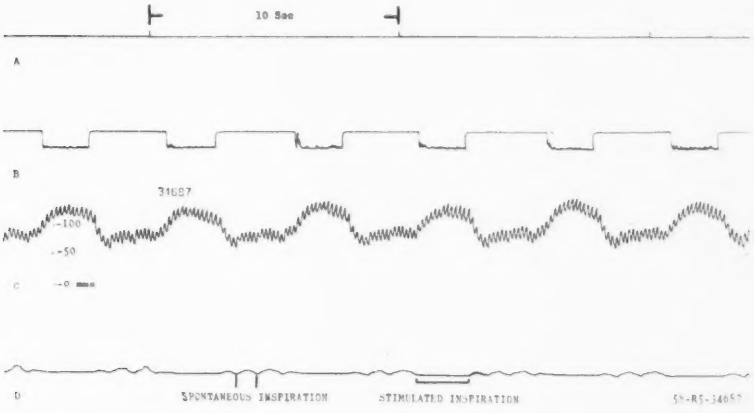

FIG. 10B. Stimulation over the heart at maximum intensity. The rise in B.P. with stimulation may be due to mechanical effects.

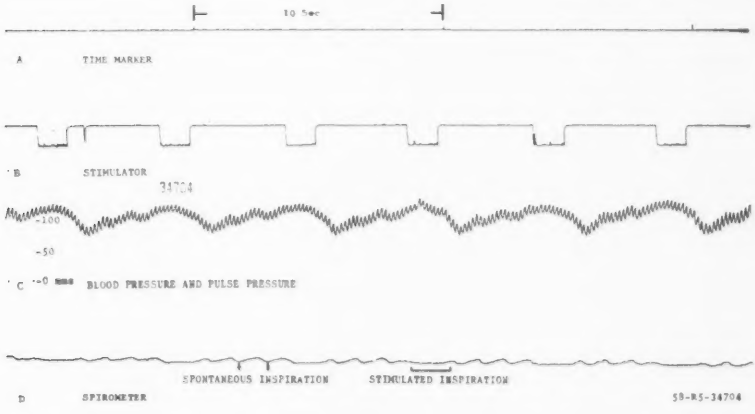

FIG. 11A. Needle electrode on the heart and normal stimulating intensity (about 40% maximum intensity). A rise in B.P. with stimulation is noted but no slowing of the heart rate can be detected.

Although no long term studies have been carried out with the type of stimulation used in these studies, it seems very unlikely that this type of stimulation would have any damaging effect on the phrenic nerve itself.

Pain. Stimulation with the Batrow stimulator produces a tingling sensation in the skin and a pulling sensation as the muscles in the area of the stimulating electrode contract. No pain is produced, and the stimulus is not particularly uncomfortable.

Explosion hazard. Since this stimulator produces a spark, it should be kept out of the operating room if explosive gases are in use.

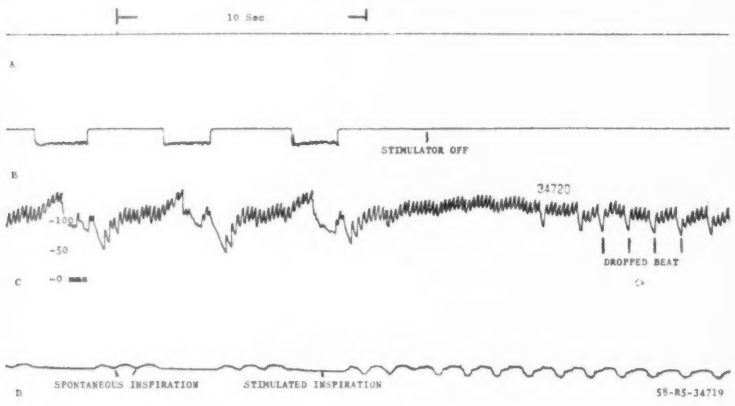

FIG. 11B. Needle electrode on the heart with maximum stimulating intensity. With stimulation there is marked slowing of the heart rate and a precipitous drop in B.P. followed by immediate recovery of the heart rate and B.P. when stimulation ceases. Eight seconds after the stimulator was shut off there was a run of dropped beats. These lasted for 30 seconds and then ceased.

SUMMARY

A new type of neuromuscular stimulator has been evaluated for possible use in newborn resuscitation. Rabbit experiments designed to determine the efficacy, mechanism of stimulator action, and injurious effects of the stimulator were carried out.

Fourteen rabbits were made completely apneic with thiopental sodium. Of these, 12 went on to complete recovery, one died after 45 minutes of resuscitation, and one died immediately after the thiopental was given.

One per cent procaine hydrochloride (0.5-1 cc) was injected into the contents of the posterior fossa of the skull of three rabbits. Four rabbits had

their phrenic nerves severed, and three rabbits were injected intravenously with succinylcholine chloride (0.5-1 mgm/rabbit). The stimulator was able to initiate and maintain respiration with severed phrenic nerves and when procaine hydrochloride was injected into the posterior fossa. However it was completely ineffective when succinylcholine was used to block the myoneural junction.

In two rabbits the stimulating electrode was placed directly on the heart and maximum stimulating intensity used. Under these conditions, cardiac slowing with a drop in blood pressure was produced. No arrhythmias were noted under normal operating conditions.

CONCLUSIONS

1. The Batrow stimulator is capable of initiating and maintaining respiration in rabbits made totally apneic with thiopental sodium.

2. Contraction of the diaphragm is achieved by direct stimulation of the phrenic nerve.

3. In order for the stimulator to cause contraction of the diaphragm the myoneural junction must be intact.

4. Fatigue of the diaphragm was noted during prolonged use of the stimulator.

5. The stimulator is noninjurious to the rabbit under normal operating conditions.

6. The stimulator is simple to use and takes only a few seconds to apply to the animal.

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THE SPECIFICITY OF THE TOXOPLASMA DYE-TEST WITH SURVEY DATA ON THE LOCAL (NEW HAVEN) AREA†

Present interest in toxoplasmosis derives not only from the clinical, pathological, and therapeutic problems presented, but also from its currently accepted status as one of the very common infections of man. Since the introduction of the dye-test in 1948,[10] most investigators in the field of toxoplasmosis have used it freely in population surveys, in research work, and, in conjunction with the complement fixation test, as an aid to clinical diagnosis. After the customary testing accorded all new diagnostic methods, it was found of world-wide serviceability and in the decade that it has been used, has come to be considered reliable, reproducible, and specific.

Recently, however, this specificity has been questioned. Cross-reactions were reported with sera from persons or animals infected with other protozoa: *Trichomonas vaginalis, Trypanosoma cruzi,* and *Sarcocystis tenella.* Whereas *T. cruzi* is found only in restricted areas, and *S. tenella* occurs very uncommonly in man, *Trichomonas vaginalis* is geographically widespread and a common human parasite. The degree of cross-reactivity reported for *T. vaginalis* was extremely high; titers considered significant being obtained in 64 per cent of infected women[8] and in 100 per cent of inoculated animals.[1]

Accordingly, the claim of nonspecificity with *T. vaginalis* required evaluation for, if valid, the toxoplasma dye-test would either have to be regarded as of limited diagnostic utility or would have to be further complicated by additional procedures in an effort to restore specificity.

This report concerns such an evaluation, made by comparing dye-test titers obtained from *Trichomonas* carriers with titers from other population groups. If *T. vaginalis* per se provokes a positive toxoplasma dye-test,

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then the sera of more persons infected with this organism should be found to have "significant titers" than sera obtained from the general population, or the trichomonas sera should show a preponderance of very high titers, or both distributions could occur.

METHODS AND MATERIALS

The distribution of dye-test titers in normal populations varies according to area, so that we first proceeded to establish a base line for normal persons in the local area (New Haven). This group of 88 normal individuals consisted chiefly of volunteer blood donors who were screened by the usual Blood Bank interrogation and were considered eligible for bleeding if they were afebrile, with normal blood-pressure and hemoglobin, suffered from no known infectious disease or symptoms thereof and were negative in the VDRL serological test for syphilis. In addition, 15 volunteers who were not donors contributed blood for this study; three of these had had laboratory contact with *Toxoplasma;* none gave titers above 1 : 1.

The *Trichomonas* group consisted of 32 females, patients of the Department of Gynecology and Obstetrics of the Grace-New Haven Hospital. Diagnosis of the infection was established by the culture of vaginal and/or cervical secretions in S.T.S. medium[7] containing 1,000 units of penicillin and 1 mg. of dihydrostreptomycin per ml.

The methylene-blue dye-test was performed in the standard manner as described by Feldman.[11] All test sera were inactivated. No serum employed as a source of accessory factor was used without a preliminary trial which indicated that 90 per cent or more of the toxoplasmata retained normal staining affinity for methylene blue after treatment with accessory factor. Saline controls, antibody controls, antigen (*Toxoplasma*) controls and accessory factor controls were routinely included in all tests.

In interpreting results, a titer of 1 : 64 was used, as more surely indicating past or present contact with *Toxoplasma* than the more customary titer of 1 : 16.

RESULTS

1. Normal population.

Eighty-eight individuals were tested in all; these comprised individuals of both sexes above the age of 21. Eighteen per cent of these titered 1 : 64 or above in the dye-test, a figure which may be taken to indicate the normal prevalence of titers at this level in adults of the New Haven area.

This rate does not fall outside the expected findings for American urban populations. Only approximate comparisons can be made with American cities since surveys by others include children and adolescents. Very probably other cities have both higher and lower infection rates.[6] In essence, within the New Haven area, ample opportunity for *Toxoplasma* infection exists, and the results recorded are not exceptional.

In the group of 88 normal individuals, 75 were aged 21-45, of which 21.3 per cent titered 1 : 64 or above. Twenty-five per cent of the 40 males titered 1 : 64 or above. Only 17.1 per cent of the females gave the same

titer, and it would be expected, from other studies, that at least 5 to 10 per cent of these females would be injected with *T. vaginalis*.[3] The difference between the sexes is not statistically significant (P=0.4) and the failure of females to predominate is not in harmony with a view holding that female trichomoniasis is a source of false positive reactions in the toxoplasma dye-test. These data are given in tabular form in Table 1 and represented graphically in Figure 1.

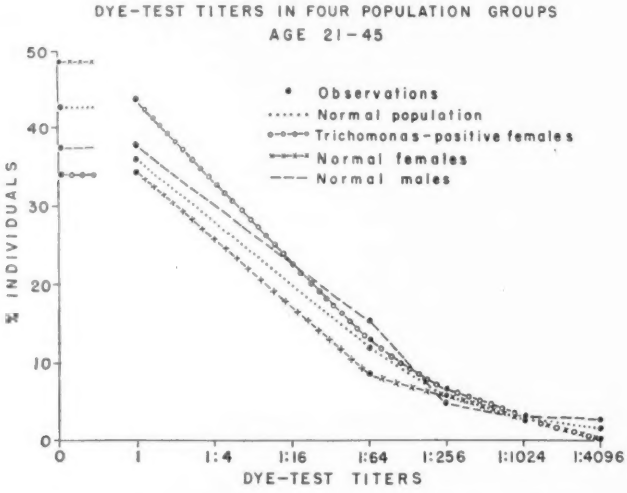

FIGURE 1. Weinman, D. and Chandler, A. H.: "The Specificity of the toxoplasma dye-test with survey data on the local (New Haven) area."

2. Trichomonas positive females.

This group of 32 women aged 21-45 were proved to carry *Trichomonas* by recovery of the organism in culture. Of these 21.8 per cent titered 1:64 or above. In Figure 1 it will also be seen that at high titers this curve approximates the distribution curves for the other groups, and thus does not indicate any predominance of high-titered sera in the *Trichomonas* group.

DISCUSSION

The incidence of *Toxoplasma* dye-tests at a titer of 1:64 or above was 21.8 per cent in the group known to be infected with *Trichomonas vaginalis*, 21.3 per cent in our group of normal blood donors of the same

age, and 17.1 per cent in a group of normal female blood donors in the same age group as the *Trichomonas*-infected women. These differences between normal and *Trichomonas*-infected females are not considered significant (P=0.6), and the investigation as a whole gives no support to the view that *Trichomonas vaginalis* causes false positive reactions in the *Toxoplasma* dye-test at titers of 1 : 64 or above.

TABLE 1. DYE-TEST TITERS IN FOUR POPULATION GROUPS

Age	*Normal population 21-45*		*Trichomonas positive females 21-45*		*Normal population females 21-45*		*Normal population males 21-45*	
Dye-test titers	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
0	32	42.7	11	34.3	17	48.5	15	37.5
1	27	36.0	14	43.7	12	34.2	15	37.5
1 : 64	9	12.0	4	12.5	3	8.6	6	15
1 : 256	4	5.3	2	6.2	2	5.7	2	5
1 : 1024	2	2.7	1	3.1	1	2.8	1	2.5
1 : 4096	1	1.3	0	0	0	0	1	2.5
Total Positive 1 : 64 and above	16	21.3	7	21.8	6	17.1	10	25
Total Individuals Tested	75		32		35		40	

Since the commencement of this investigation several papers on the same subject have appeared. Cathie[4] compared the titers obtained from 60 women with proven trichomonas vaginitis with two series of controls totalling 309 females. He found that "In the cases presented here there is no material difference in the positive dye test findings in cases with known trichomonas infection and those without." Four men infected with trichomonas gave negative dye tests.

Borgen and Björnstad[5] reported on the sera of 13 patients suffering from severe and long-lasting *Trichomonas vaginalis* vaginitis all reacted negatively in the dye test.

Feldman and Miller,[6] over a period of years, dye-tested 1191 normal persons; when these were divided into reactors of the two sexes producing

titers at 1 : 16 or more, the rate for males was 35.9 per cent; for females 35.6 per cent. This does not indicate that *Trichomonas vaginalis* infections in women provoke false positive reactions and is not in harmony with the results recorded by Michalzik.

Michalzik[3] reported that 64 per cent of 50 women with *T vaginalis* gave dye-test titers of 1 : 25 or higher. For comparison this author cited rates found by others in the normal population of other German cities. The highest rate cited was 18 per cent positive in Bonn at titers of 1 : 20 or more. Thus, at least three times as many infected women should yield significant titers in the dye test as does the general population. This would indeed indicate a cross-reaction; however, as seen above, confirmation of the finding has not been forthcoming. The discrepancies in the results obtained by different investigators is striking; had Michalzik provided a group of controls whose titers were determined by the same personnel using the same techniques, an explanation might be available.

Awad[1] reported that rabbits and mice inoculated with bacteria-free *T. vaginalis* all subsequently yielded sera reacting in the toxoplasma dye-test at high titers. For example, of 12 mice inoculated, the titers were 1 : 128 in two animals, 1 : 256 in three, and 1 : 512 in the remaining seven. Borgen and Björnstad[2] repeated the mouse work and all 10 mice reacted negatively.

Finally, it may be worth mentioning that the alleged dye-test cross reaction between *Sarcocystis* and *Toxoplasma* also has not been confirmed in two independent investigations.[5, 9]

SUMMARY AND CONCLUSION

1. In the New Haven area, 21.3 per cent of the normal, healthy adult population has given evidence of contact, past or present, with *Toxoplasma.*

2. In the same area, 32 adult women infected with *Trichomonas vaginalis* gave a 21.8 per cent rate utilizing the same method, technique, and criteria.

3. These results do not confirm a previous report of nonspecificity of the Toxoplasma dye test based on a three-fold or greater incidence of significant titers in *Trichomonas*-infected women than in the general population.

4. Since critical investigations by others also indicate no cross-reactivity between *Toxoplasma* and *Trichomonas* there is no present reason to believe that any such relation does in fact exist.*

* One other recent paper, seen after presentation of this article for publication, states that there is no difference in the frequency of positive dye tests in women who are infected with *Trichomonas* and those who are not (Piekarski, G., Saathoff, M., and Korte, W. *Z. Tropenmed. Parasit.,* 1951, *8,* 356-367).

5. The local incidence rates for Toxoplasmosis are consistent with values reported for certain other American cities. The reasons for the low case-report rate remain to be determined.

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PATTERNS OF ENAMEL PROTEIN ELEMENTS IN THE MOUSE INCISOR

INTRODUCTION

Protein elements have been known to exist in enamal, but their structure and role in the final formation of enamel have not always been agreed upon.[2, 7, 8, 9, 11, 12, 14, 15, 16, 17, 18, 24, 25, 26] In 1933 a "ladder-like" process was described by Kitchen in relation to newly formed enamel in the mandibular incisor of the white rat.[10] Previously, Bibby reported an increase in the organic elements of hypoplastic enamel which he believed resulted from consolidation of the rod sheath spaces.[2] Rosebury had also described the protein structure of enamel.[17] He felt it represented remnants of the living structure of the enamel organ which, as he stated at that time, added nothing to the development of enamel except perhaps its toughness and elasticity. It was noted by Sognnaes in studies on the organic elements of the teeth that an organic framework existed between the external and internal enamel matrices which was similar in both decalcified and ground sections.[19, 20, 21] With a delicate sudanophilic reaction and a toluidin blue stain, Sognnaes demonstrated the formation of these prism sheaths and their interprismatic structure.[22] Much more recently Gustafson described the interior of prism sheaths of enamel rods and showed an organic framework divided or arranged in regular spaces within the sheaths.[8] It is Helmcke's contention that prism sheath boundaries as such do not actually exist, but are merely the result of directional differences of similar and smaller organic patterns arranged between each developing prism.[9] Helmcke's electron microscopic investigations amplified the previous ideas of Lams, who stated that there were no definite limiting membranes between enamel prisms, but there were short protoplasmic bridges passing from one prism to another.[11] The limiting membranes of the ameloblasts, Lams stated, were very delicate and scarcely stainable, being much more like condensations of peripheral material.

From the above it has been established that prior to mineralization and calcification a protein pattern is formed and arranged more or less in an orderly fashion inside and between each prism, and that afterwards the

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inorganic parts of enamel are laid down in the form of crystallites within these organic meshes or spaces.[1, 8, 9, 26] In addition, the formation of the organic meshwork is dependent upon the metabolic activity of the ameloblasts.[12, 15]

Studies have usually been concerned with the inorganic aspects of enamel formation, and when the organic matrix was considered, attempts were not made to compare its histological structure under different experimental conditions. It is the purpose of this communication to compare the effects of NaF, CaF_2, and Na_2SiF_6 in concentrations of 1:100,000 by weight or 10 ppm on the organic matrix of enamel in the mouse mandibular incisor. While it is accepted that fluorides are involved in certain of the metabolic processes of formation of the hard structures of the teeth,[6, 7, 25] their actual role remains enigmatic. No previous histological investigations have dealt with the comparative effects of these three different fluoride compounds on the organic matrix of enamel. Mandibular incisors are ideally suited for such an investigation of enamel matrix formation, for, as continuously growing teeth, all stages of amelogenesis are at once observable.

METHODS

Young adult dba Rockland mice of similar weights and ages, and equally divided as well as separately maintained according to sex, were used for these experiments. Only the mandibular incisors were studied for this report.

These experiments were conducted under the following conditions:

1. Mice that received only tap water.
2. Mice that received distilled water *ad libitum* for 42 days.
3. Mice that received NaF, CaF_2 and Na_2SiF_6 in 1:100,000 concentrations by weight in distilled water *ad libitum* for 42 days.

Animals were maintained in groups of five males and five females under each of the above categories for the duration of these experiments. Fluid intake was measured daily from graduated drinking bottles and amounts recorded. Animals' weights were recorded three times weekly. Feeding was *ad libitum* with Purina Laboratory Chow.

For the periods of these experiments, the average fluid intake of each mouse was about 3 cc. per day. There did not appear to be any significant variation between the different categories with regard to amounts consumed. The greatest increase in weights took place where the animals received CaF_2.

Sacrifice of animals was by chloroform inhalation. Mandibles were immediately removed, separated, freed of adhering flesh, and placed in Zenker's solution. After 12 hours they were removed and washed in tap water for 24 hours. They were then placed in 80 per cent alcohol for 24 hours before decalcifying. Sectioning was usually about 7 microns and staining was with hematoxylin and eosin.

Each mandible half was sectioned vertically through the midline so that the entire length of the ameloblasts and the enamel matrix that remained after decalcification was presented for histological study. Additional jaw halves were cut in serial cross section so that matrix formation could likewise be examined from this aspect. Direct and

refracted light, usually under oil immersion, were used to study the organic matrix of the mandibular incisors under the several conditions of these experiments.

An entire series of these experiments was repeated 20 times; in addition another entire group of experiments were similarly conducted in which concentrations of the fluoride salts were at 20 ppm. In still other experiments, comparisons were made between fluoride salts dissolved in tap and distilled water: in these, no significant histological differences could be observed.

FINDINGS

The organic matrix that remains following decalcification in the mouse mandibular incisor is comprised of three distinct zones. They are, for reference and for the reader's orientation, designated by this investigator as Zones A, B, and C. They will be described below and hereafter will be referred to by these headings. These zones consistently appear at particular places and in a constant relationship to each other in the mandibular incisors of these mice, when longitudinal sections are cut along the midline for the entire length of the enamel protein matrix. These zones are described as follows and are shown in Figure 1:

Zone A, the secretory zone, located at the base of the incisor and extending incisally to Zone B. Ameloblasts are actively forming the protein matrix and lie at an angle of about 145° to it.

Zone B, which is between Zone A and Zone C, and is represented by the gap between the ameloblasts and underlying enamel matrix. This is where the ameloblasts and the outer border of the matrix have become altered. This zone is called by this investigator, "The Zone of Compression."

Zone C, which is adjacent to Zone B and extends toward the incisal tip. This is the zone of reattachment where the ameloblasts rejoin the underlying matrix and regain their length.

In Zone A the ameloblasts, as matrix-secreting cells, lie in a direct association with the patterns of organic fibrillar material that they are delivering in successive increments. Figures 2-6 are representative sections of this secretory zone as observed under the five different categories of these experiments. The ameloblasts are to the left of the center in these figures, the organic matrix occupies the center or main portion, and to the right the dentin border is approached. Near the dentin the structure becomes more homogeneous and patterns in this area may be obscured by the deep hematoxylin stain. However, towards the left, a fibrillar structure of "herringbone" appearance is continuous with and involves the Tomes' processes of the ameloblasts. The entire region, from the basal aspects of the ameloblasts to the dentin, varies in width and length as well as in staining under the several conditions of these experiments.

In tap water control animals as represented in Figure 2, compartments of the matrix were not always secreted in close apposition to each other. Where distilled water had been given, as shown in Figure 3, matrix formation was heaviest, stained darkest with hematoxylin, and was narrowest in width when compared with similar sections from the other categories. There was a similarity in the regularity of the enamel protein matrix where NaF and Na_2SiF_6 were used that was not observed in other experiments. However, with Na_2SiF_6 the ameloblasts appeared to be less numerous in the secretory zone and perhaps because of this the patterns did not lie close to each other. Where CaF_2 was administered, matrix formations were wider, shorter, and took more of an eosinophilic than a hematoxylin stain. When CaF_2 was used, Zone A was markedly increased in width. In addition, as shown in Figure 5, there were more prominent concentrations of secretory material lying between the Tomes' processes and the site of formation of the enamel protein matrix. With Na_2SiF_6-treated animals there were frequently found irregular globular concentrations or formations deep within the organic matrix near the dentin margin as seen in Figure 6.

Zone B is indicated by an abrupt gap or space between the ameloblasts and the underlying enamel matrix. This is not an artifact for it is consistently observed in the mandibular incisors of mice. The fibrillar pattern or compartmental network which assumed a regular direction in its entirety in Zone A is now changed. The outermost margins have altered their direction, while the center or main body retains its original plane or direction. This main part always slants at an angle of approximately 45° towards the base or growth center of the incisor; thus the location of the base or tip of these teeth can be determined from the direction of these compartments. Ameloblasts in Zone B are also altered in position and appearance because of three conditions peculiar to this region. These are: 1. accumulation of numerous vacuolar or intercellular spaces that push or wedge between the ameloblasts' polar aspects; 2. separation of ameloblasts from the underlying matrix; and 3. compression, presumably from accumulation of fluid materials in this area, so that the ameloblasts become shortened in appearance. Presumably, also from compression, the matrix was depressed in this zone and this indented region varied in depth according to the experimental treatment. Upon the matrix surface in Zone B, there was consistently found an irregular cluster of acellular, light eosinophilic material which varied considerably in amount. Under certain conditions, as with distilled water, Zone B was located nearer to the incisal tip when matrix formation was relatively narrow. When the matrix formation was wider than usual, as with CaF_2, Zone B was located nearer to the basal

part of the incisor. Where distilled water had been given, as shown in Figure 8, Zone B was smallest. Compression of the matrix and of the basal aspect of the ameloblasts was least in such cases. Zone B was largest in comparison with the others where NaF had been given, as demonstrated in Figure 9. Both with NaF (Fig. 9) and with CaF_2 (Fig. 10) compression of the fibrillar matrix was well marked, and in such instances there appeared to be greater accumulations of the light staining acellular material on the surface. With Na_2SiF_6, however, there was not the well-marked indentation of the matrix that was observed with the other fluorides. Aggregations of vacuolar areas that wedged between and distorted the nuclear ends of the ameloblasts were larger and more numerous in all fluoride-treated incisors than in control incisors. This is shown in Figures 9 through 11.

It has been indicated previously that Zone C is the most anterior of the three zones, and it is in this region that the ameloblasts and underlying matrix reassume their relationship, but in a different manner than was observed in Zone A. In Zone C the matrix begins to stain progressively lighter, ultimately terminating in a very light staining fibrillar pattern. This ends where the entire enamel structure, under the present experimental conditions, is lost by decalcification. It is in this lighter staining area of Zone C that cross sections of the incisors were taken, as shown in Figures 17-22. Close to its junction with Zone B, Figures 12-16 show Zone C, where ameloblasts have initially become reattached to the subjacent matrix. These figures are in longitudinal midsection of the incisors, the same as the preceding eleven figures. The Figures 12-22 differ, however, in that these were all taken with the phase microscope.

The organic framework or fibrillar pattern of Zone C, as it lies between the dentine and ameloblasts, is divided into three areas. These are: a narrow deep or internal part, a wide center or main part, and an outermost region that is usually about one-fifth to one-fourth of the entire matrix in width. In the cross section (Figs. 17-21), these regions are completely seen.

A difference in the directional plane in the outermost part of the matrix was apparent in Zone C (Figs. 12-16) near the junction with Zone B, and according to the experimental conditions this varied in extent or depth. The center or main part of the organic fibrillar matrix in Zone C had the same directional plane and compartmental formation that was formed in Zone A. The difference in Zone C was, however, that while the main or center part of "ladder-like" formations remained unchanged, the outer and inner regions were altered in appearance. The outer part was changed in direction and the inner part appeared to be slightly compressed

against the underlying dentin. As in Zone A, it was likewise apparent in Zone C that irregular patterns appeared between the orderly compartments whenever they were not in close apposition. This was evident with the tap water controls, as shown in Figure 12. Furthermore, as previously observed in Zone A, matrix patterns in Zone C were more regular, as compared with the other categories, in size and shape, where NaF (Figs. 4 and 14) or Na_2SiF_6 (Figs. 6 and 16) had been given. In addition with Na_2SiF_6, there were more interruptions as compared with NaF, between the precise compartment formations. This was previously noted in Zone A. Where distilled water had been given, as shown in Figure 13, matrix compartments were heavier and stained much darker than in the other instances. There was also an alteration in the staining reaction with CaF_2 (Fig. 15), for in such instances the stain was much lighter.

Figures 17-22 are cross sections of the incisors, taken at right angles to the previous longitudinal sections. They reaffirm the preceding observations with regard to the enamel protein matrix in these experiments, namely: that patterns were not consistent with tap water (Fig. 17); that fibrillar patterns were heavier, stained deeper, and were less compressed with distilled water (Figs. 18 and 22); that compression was greater with NaF and CaF_2 (Figs. 19 and 20); that staining was lighter with CaF_2 (Fig. 20); and that the patterns observed with NaF and Na_2SiF_6 presented certain common features (Figs. 19 and 21).

DISCUSSION

This presentation demonstrates that basic alterations can be induced in the fibrillar protein matrix of enamel as formed in the mandibular incisors of mice. The initial steps in enamel prism formation result from the secretory activity of ameloblasts.[10, 11, 12, 15, 25] Like other gland cells, they maintain a definite relation to their excretory products.[1] In the present experiments, this relationship is illustrated in the Zones that have been designated as A, B, and C. Following matrix formation, minerals are laid down within the spaces or compartments in the form of crystallites, usually with their long axes in the direction of the prisms.[8, 12]

This sequence of events is not held by Scott, who feels that the matrix, when first laid down, does not have a pattern, but is amorphous or homogeneous.[18] The present studies, however, do not support this view, for a definite pattern in Zone A can be observed as the matrix is being delivered in successive increments. The compartments of the organic matrix may differ in size, shape, and arrangement and thus affect subsequent arrangement of crystallites, which in turn would affect the final structure of calci-

fied enamel. In addition, the structure of enamel would also depend upon the number of active ameloblasts and the duration of their secretory activity. Therefore, where large numbers of ameloblasts were maintained and their secretory activities prolonged, as with tooth germ transplants in female guinea pig castrates, the extent and amounts of matrix formations were increased, and tooth development was enhanced.[4]

The more orderly and regular matrix pattern following NaF administration, as seen in the present study, may help to explain one possible factor regarding the effects of low concentrations on the teeth. At the same time, while considering this concept, it is also important to note that there was a difference in patterns and also in staining with the other substances used in this study. However, the fibrillar pattern seen in instances where Na_2SiF_6 had been used, more nearly approached that pattern observed with NaF.

Heavier matrix formation observed when distilled water was administered should have prevented crystallites from being deposited within the matrix spaces to the same extent and regularity as they were deposited when these spaces were larger and more uniform. Each compartment or space, as pointed out by Gustafson,[8] represents a center of mineralization and calcification. The total areas or volumes of the compartments formed where distilled water was given were much less, due to the greater thickness of the matrix; thus enamel formation would not or could not have the same crystallite orientation attained under the other experimental conditions. This is also in accord with Bibby's observations with hypoplastic teeth, in which he found that the amount of organic matrix was increased.[2]

Another factor in Zone A which had an effect on pattern formation was that where there were fewer ameloblasts with tap water and with Na_2SiF_6. As a result, there were more interruptions between the regular compartments of secretory material. Such interruptions or spaces, as observed in the present study, contained less orderly arrangements of fibrillar material. This naturally raises a question concerning the views of Helmcke and Lams,[9,10] for they felt that the smaller and regular compartments which filled these interprismatic spaces were in a different plane from the main part of the prism formations. The formations between the prisms, as seen in this study, could have been in a different plane, but their regularity certainly was not remarkable. It is also possible that as Zone B was reached, compression in this area caused a considerable reduction of the interprismatic spaces.

The basic alteration in the organic matrix, where CaF_2 was administered, may have resulted from an increased absorption of calcium from the gut.

In dealing with these fluoride compounds at the concentrations used, any difference in effects may have been on a basis of inorganic ion exchange. Differences in the physiological effects of these compounds may have been related to an upset in the intra- and extracellular ratio of cations and anions, to competition between cations or anions (Ca^{++} or F^{-}) with Mg^{++}, or to inhibition or activation of high energy phosphate conversion by competition or antagonism of cations for places on the enzyme protein.[5, 6] Such factors may have accounted for the increased concentrations of secretory material that accumulated between the ameloblasts in Zone A where CaF_2 and NaF had been administered. Later, when calcification of the matrix is supposed to take place, these factors could also have resulted in an upset of this process, for any effort by the tissues to bind Ca^{++} with PO_4^{---} or as calcium-apatite in attempting to restore the Ca^{++} relationship to Mg^{++} may be impossible as long as F^{-} is present. Therefore, calcification of the protein matrix would not take place or would be greatly retarded.

Marshland has referred to the area designated at Zone B as an artefact, but he does state that where it begins the secretory zone ends.[12] He continues, "The artefact now disappears and the ameloblasts, now reduced in size, become reattached to the matrix surface." This zone of reattachment has been designated as Zone C in this report.

Several factors about Zone B should be discussed with regard to the fibrillar pattern of the organic matrix. It is at the beginning of this region that the outer border of the matrix is slanted towards the incisal tip. It also appears as though an increased pressure, presumably fluid, is built up here. During this increase in pressure, the basal aspects of the ameloblasts become compressed, as well as the matrix opposite them. Thus, a more applicable term than Zone B would be the "Zone of Compression." Sognnaes, in quoting Gustafson with regard to the organic elements of enamel, states that there may be bends or changes in prism direction during growth and these directional changes are caused by traction.[20]

A number of years ago, Boedecker observed that there were two kinds of enamel—one which had a regular mosaic pattern, and the other a gnarled enamel.[3] He felt that enamel became gnarled because of stress, either at the occlusal surface or at the dentino-enamel junction. In his investigations on the eruption rate of the rat incisor, Sturman found that considerable pressure or traction was involved, which could be determined by studying the fibers of the periodontal membrane.[23] In the present study, the force of eruption plus pressure created at Zone B must have been responsible for directional changes taking place in the outer layers of the matrix, and it would be of basic interest to study eruption rates in rats

and mice with comparison to the position of Zone B. It seems likely that the farther away from the base of the incisor Zone B is located, the slower the eruption rate. Thus, distilled water animals should have the slowest eruption rate. In addition the size of Zone B, in relation to eruption rates, should also be considered.

Except for the alterations taking place in Zone B, the matrix pattern in Zone C is consistent with that observed in Zone A. In this area, the reattachment of the matrix to the ameloblasts did not seem as firm and was often not maintained where animals had been given distilled water. It is thus apparent that the firm reattachment taking place in the area we have designated as Zone C, as consistently observed by Marshland,[12] is subject to variations under different experimental conditions.

The question naturally arises as to the direction of prisms which consistently appear to be oriented in particular planes at an angle of about 145° with the ameloblasts. This was apparent in Zone A and remained so in Zone C. Interruptions with this regular pattern appear to be readily achieved, and once formed in an altered manner are not easily changed during the succeeding phases of matrix maturation. Initially, the ameloblasts form a fibrillar matrix pattern in regular and successive increments, which may be interfered with.

Another interesting fact brought out in Zone C is the consistency of matrix formation. Sections cut at right angles to each other in the upper limits of Zone C bear this out, for there is a uniformity of the matrix pattern, whether it is cut longitudinally or in cross section. Marshland speaks of a loss or change in the pattern of the matrix as maturation takes place, and that further calcification of the matrix by removal of excess water and organic matter causes this change.[13] If we accept the earlier views of Lams[11] and the more recent findings of Gustafson,[8] Sognnaes,[22] and Helmcke,[9] then Marshland's views[13] need further explanation. Furthermore, the findings in the present study with regard to matrix formation emphasize that alterations in the pattern of the matrix would likewise alter the direction of crystallites in the mature enamel.

SUMMARY

In the mandibular incisors of mice, the ameloblasts secrete a fibrillar matrix of organic material that has a definite pattern which may vary according to certain of the experimental conditions used in this study. This region is known as the secretory zone and is designated as Zone A. As this fibrillar matrix moves incisally, it undergoes certain structural changes in Zone B, which is the "Zone of Compression." The outer por-

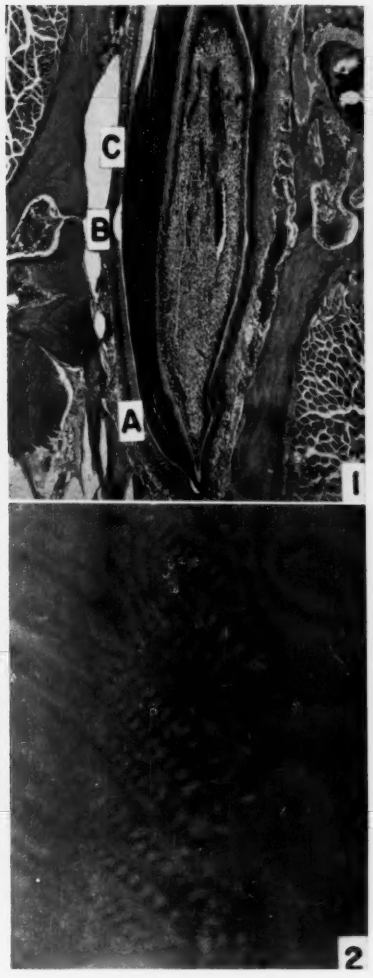

FIG. 1. Regions of the enamel organic matrix and zones designated as A, B, and C. From mandible of male mouse that received CaF_2 *via* its drinking water. Original magnification x25.

FIG. 2. Zone A, the matrix formative zone, in tap water control adult male mouse. Ameloblasts to the left border and matrix formation in the right two-thirds of photomicrograph. Original magnification x1745, oil immersion.

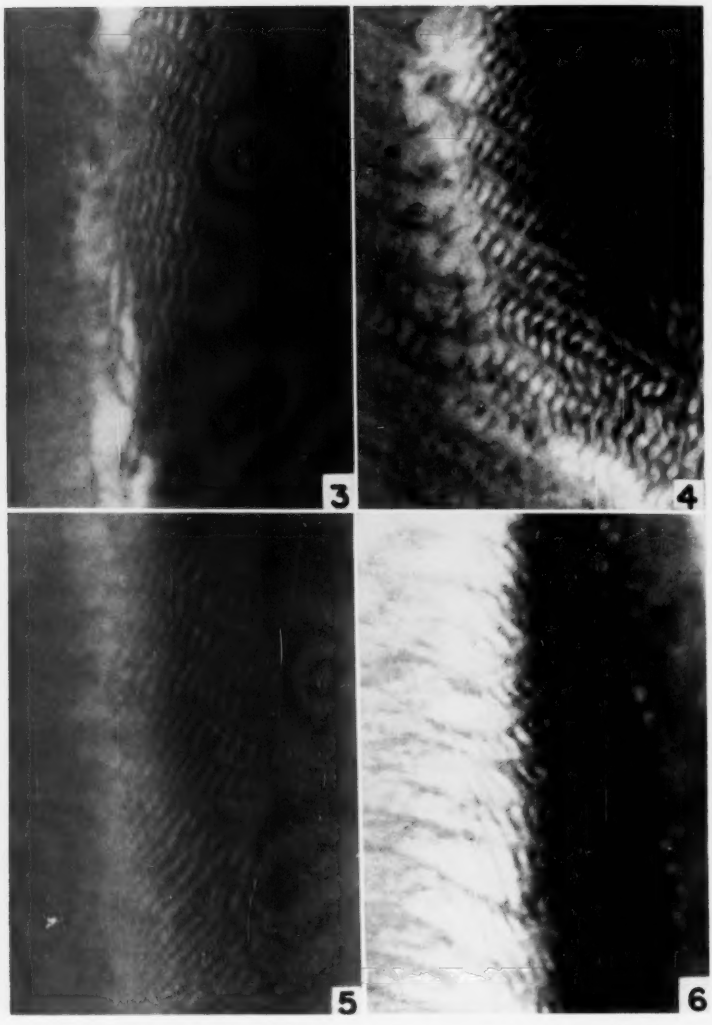

FIG. 3. Zone A, the matrix formative zone, in a mandibular incisor of male mouse that received distilled water as drinking water for 42 days. Ameloblasts to left and remaining two-thirds to the right is the matrix formation. Original magnification x1745, oil immersion.

FIG. 4. Zone A in a female mouse that received NaF *via* its drinking water for 42 days. Ameloblasts to left and in remaining two-thirds to the right is matrix formation. Original magnification x1745, oil immersion.

FIG. 5. A matrix formation in female mouse that received CaF_2 *via* drinking water for 42 days. Ameloblasts to left and remaining two-thirds shows matrix formation. Original magnification x1745, oil immersion.

FIG. 6. Matrix formation in male mouse that received Na_2SiF_6 *via* drinking water for 42 days. Ameloblasts to left half and the remainder, matrix formation which borders on dentin along extreme right border. Original magnification x1745, oil immersion.

FIG. 7. Zone B, a tap water control from a single mouse. Center left enamel matrix formation, center right ameloblasts. Original magnification x240.

FIG. 8. Zone B in a male mouse that received distilled water for 42 days. Center shows matrix of enamel and center-right shows ameloblasts. Original magnification x240.

FIG. 9. Zone B in a male mouse that received NaF *via* drinking water for 42 days. Diagonally in left center is enamel matrix and diagonally to the right of center is ameloblastic layer. Original magnification x150.

FIG. 10. Zone B in a male mouse that received CaF_2 via its drinking water for 42 days. Center left enamel matrix, center right ameloblasts. Original magnification x240.

FIG 11. Zone B in a female mouse that received Na_2SiF_6 via drinking water for 42 days. Center enamel matrix and to the right of center ameloblasts. Original magnification x200.

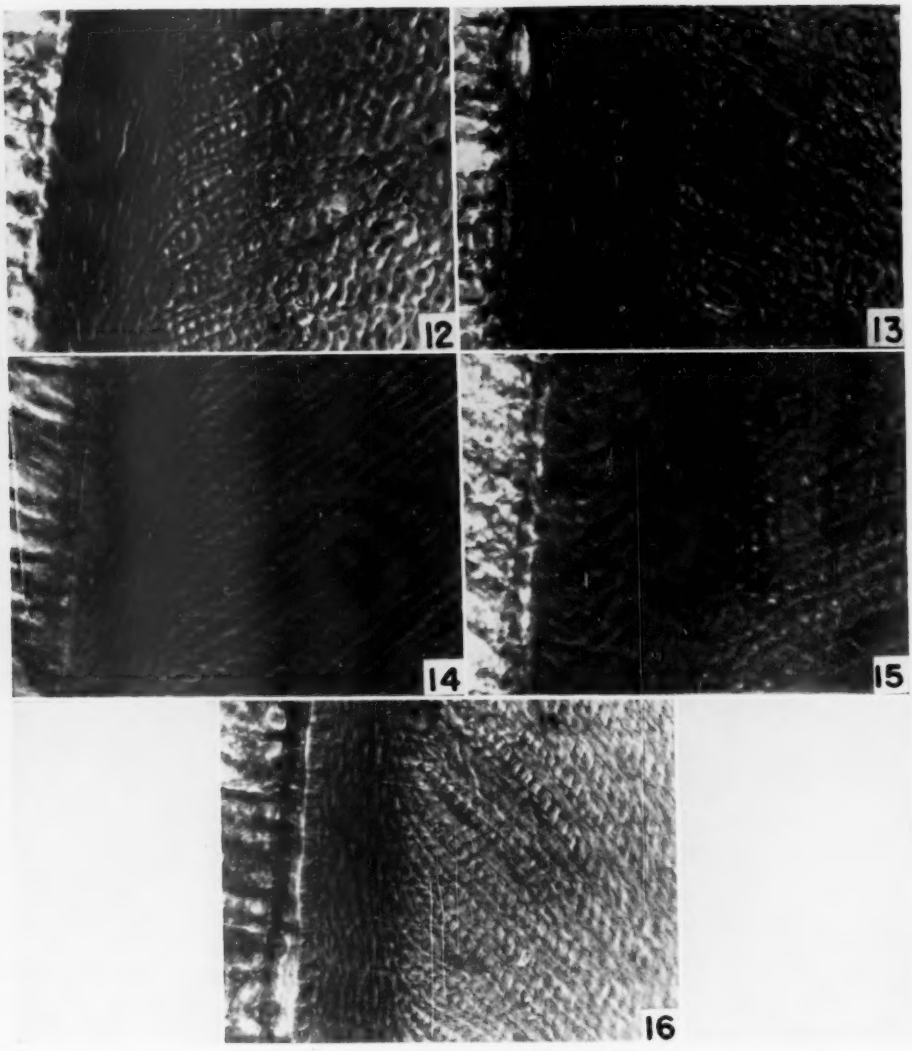

FIG. 12. Zone C of a female mouse used as a tap water control. Ameloblasts to left border. Original magnification x1900, phase oil immersion.

FIG. 13. Zone C. The male mouse received distilled water for 42 days. Ameloblasts on edge of left border. Original magnification x1900, phase oil immersion.

FIG. 14. Zone C in a male mouse that received NaF water for 42 days. Ameloblasts on left edge. Original magnification x1900, phase oil immersion.

FIG. 15. Zone C. The female mouse received CaF_2 for 42 days. Ameloblasts to left border. Original magnification x1900, phase oil immersion.

FIG. 16. Zone C. The female mouse received Na_2SiF_6 for 42 days. Ameloblasts on left border. Original magnification x1900, phase oil immersion.

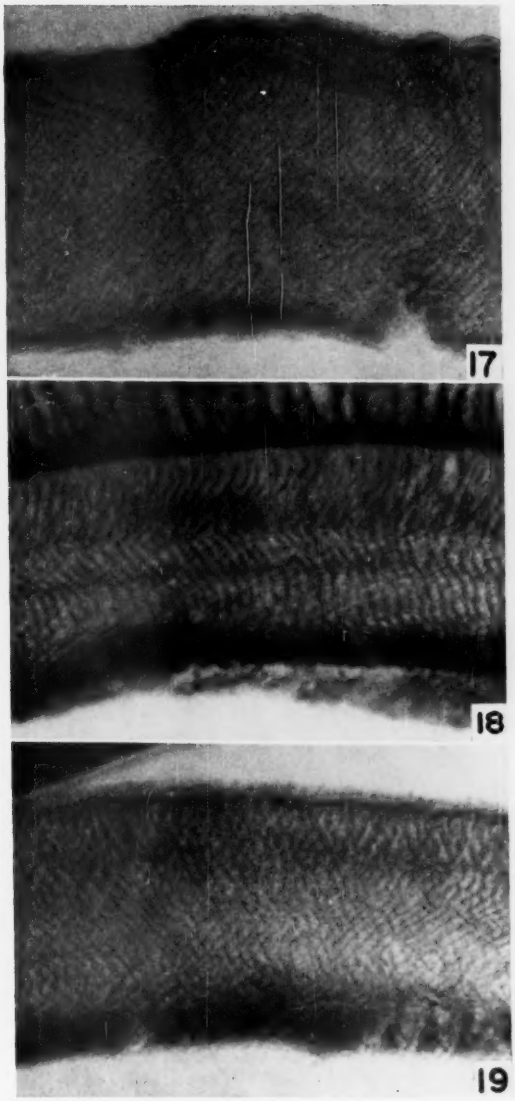

FIG. 17. Extreme limit of Zone C toward incisal edge. Male mouse, tap water control. Original magnification x1900, phase oil immersion.

FIG. 18. Extreme limit of Zone C toward incisal edge from female mouse receiving distilled water for 42 days. Original magnification x1900, phase oil immersion.

FIG. 19. Extreme limit of Zone C. A female mouse received NaF for 42 days. Original magnification x1900, phase oil immersion.

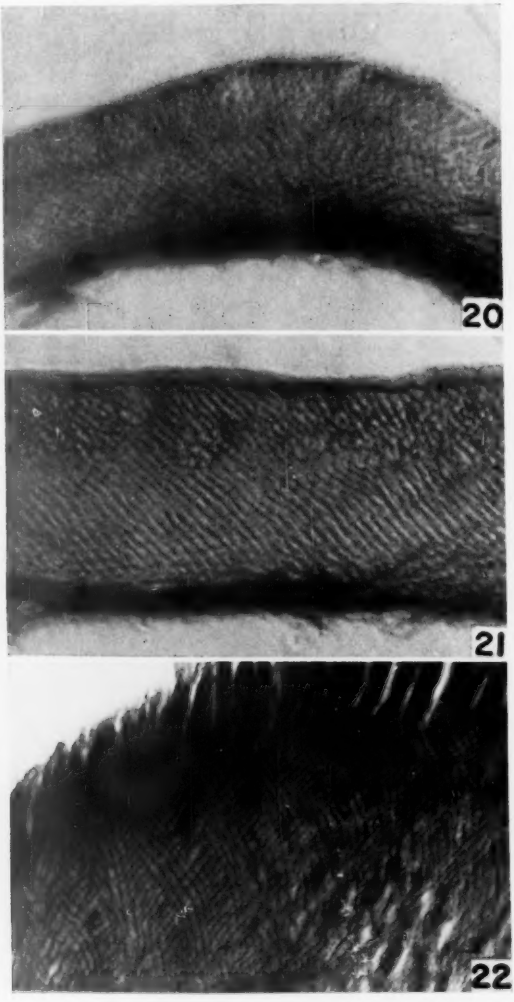

FIG. 20. Extreme limit of Zone C. A male mouse received CaF_2 for 42 days. Original magnification x1900, phase oil immersion.

FIG. 21. Extreme limit of Zone C. A male mouse received Na_2SiF_6 for 42 days. Original magnification x1900, phase oil immersion.

FIG. 22. Zone C. Extreme limits where a female mouse received distilled water for 42 days. Note heavy pattern of matrix. Original magnification x2000, phase oil immersion.

tion of the matrix is changed in direction while the main or central portion retains the directional plane assumed in Zone A. The inner portion, which lies against the dentin, also undergoes changes which appear to result from compression of the deeper fibres. Varying amounts of acellular material appear on the surface of the matrix surface in Zone B. Ameloblasts and the matrix surface also undergo certain changes. The ameloblasts become shortened and change their shape and the matrix surface becomes depressed. The size of Zone B and the degree of compression of the matrix and ameloblasts differed under particular experimental conditions. It is felt that in Zone B, minerals in form of crystallites are forced into the spaces or compartments of the matrix and with each space acting as a center of mineralization, the pattern of the matrix thus influences the ultimate structure of calcified enamel. As Zone C is reached, the ameloblasts become reattached to the matrix and it now appears that whatever force caused the ameloblasts to separate from the matrix in Zone B has now disappeared.

The matrix pattern achieved in Zone A, with the exception of the outer and inner peripheries, is maintained throughout Zones B and C. Closeness of the series of "ladder-like" compartments to each other appears to depend upon the actual number of ameloblasts engaged in the secretion of the matrix in Zone A, and the forces that maintain them or push them in close apposition as the entire mass moves incisally. This may also involve factors that are concerned with tooth eruption. Events taking place here, however, may be affected by different experimental conditions. Under favorable conditions the regularity, the plane of prism direction, and the apposition of patterns to each other gives the appearance of a continuous series of compartments of fibrillar material of equal size and in a single plane of direction. When conditions are unfavorable, less well-organized patterns appear between the regular series of compartments for varying distances.

These patterns of the fibrillar matrix of enamel varied under the several conditions of these experiments. It was most regular with NaF, heaviest with distilled water, and widest with CaF_2. In the tap water controls and also with Na_2SiF_6, interruptions in the regular formations of compartments were more frequent. Concentrations of the fluoride salts were all at 1:100,000 by weight in distilled water, and animals were maintained on them and also on distilled water and tap water for 42 days before sacrifice. Staining was deepest with hematoxylin where animals had received distilled water, and where they had received CaF_2, it was lightest.

The area designated as Zone B varied in length, being smallest when mineralization was apparently poorest, as with distilled water animals.

When the matrix was narrowest, as with distilled water, Zone B was located nearer towards the incisal tip. When the ameloblasts and matrix appeared to be greatly compressed, Zone B was largest and this was observed with both NaF and CaF_2.

In Zone C, except in the inner and outer peripheries, the matrix retained the regular pattern that it achieved in Zone A. This was true whether the matrix was sectioned in longitudinal midsection or in cross section. The peripheral changes in direction that became evident in Zone B were also evident in Zone C and presumably came from pressures or traction first apparent in the preceding zone.

CONCLUSIONS

1. The fibrillar protein matrix of the mouse incisor which remains after decalcification has three distinct areas which are designated as follows:

   Zone A—the zone of secretion
   Zone B—the zone of compression
   Zone C—the zone of reattachment

2. The fibrillar matrix which is formed in successive increments by the ameloblasts and delivered by the Tomes' processes may vary under different experimental conditions.

3. The matrix is formed at a definite angle to the ameloblasts and its compactness depends upon the number of ameloblasts actually engaged in secretory activities in Zone A.

4. Where NaF and Na_2SiF_6 were given, the compartmental spaces were more regular.

5. Where distilled water had been given, the matrix was much heavier.

6. Where CaF_2 had been given, the matrix was wider.

7. The size of Zone B was greatest where NaF and CaF_2 had been given.

8. The pattern of arrangement did not change after formation in Zone A except along the inner and outer peripheries, and such changes were due to forces of eruption and to the pressure created in Zone B.

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THE USE OF MONOZYGOUS TWIN CASES IN THE STUDY OF PSYCHOSOMATIC CONCEPTS†

Workers seriously interested in genetic problems have long deplored emphasis in the clinical literature on case reports concerning disease occurring in both members of a pair of identical twins (concordant). The value of reporting cases of illness appearing in one member of a pair (discordant) is obvious, yet such reports appear only sporadically. Increased attention to the role of the family and society in the genesis of schizophrenia has given new prominence to the study of mental disease in twins. Case reports are now appearing with considerable detail. This, interestingly enough, has been paralleled in medical research by an increased awareness of the significance of twin disease, in part because of the results of organ transplants in identical twins. It is the purpose of this paper to discuss some of the problems in evaluating twin case reports and to emphasize the value of the study of twin cases in the examination of psychosomatic concepts.

The methodology of twin study deserves some comment. At first it might seem that the chief value of case reports of discordant disease in twins would be to lend support to hypotheses concerning the environmental (or at least the "other than genetic") factors in the genesis of disease. As will be shown later, this cannot be accomplished by finding one discordant case. The statistical techniques introduced by Kallman have limited usefulness in establishing the genetic background of disease, but cannot assess the importance of the assorted factors that make up "environment."

In controversies over such issues, the suggestion of study of identical twins reared apart from birth arises. Early studies were not rewarding from the viewpoint of modern personality theory; their chief value has been a negative one: to demonstrate that many human attributes are

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significantly less variable in identical twins. The case material of Newman[9] and of Burks[1] suffers from the fault that in most cases the early environmental differences of the separated twins were not great, and where personality differences did exist, the family situation, the characteristics of the parents, or the recall of early experiences, were not sufficiently detailed to allow interpretation. In addition, the tools of projective psychological testing were not used to full advantage.

Price[8] has questioned whether the assumption that dissimilarity in monozygous twins is as important evidence for the primacy of environmental circumstances as it might first appear. For example, intrauterine disadvantages of circulation or position may be a prenatal cause of differences apparently developing in later life in monozygous twins. Therefore, he has suggested that only monozygous twins with equal (dichoroidal) placental circulations be studied, since in such patients circulatory changes would be less likely to produce differences which might be interpreted as the effect of post-natal environment. Price estimates that dichoroidal circulation is found in 35-40 per cent of all monozygous twins. Such a suggestion has obvious merit, but lack of information about the placenta would sharply limit the twin material available for study.

From a psychoanalytic standpoint, objections can be raised to most twin studies; unless one has an opportunity to examine the subtleties of the developmental years of early childhood in great detail, or to recover the framework of such development by means of therapeutic analysis, the significance of later physiological and psychological data is debatable.

If the limits suggested by Price were followed closely, very little twin case material would be available. It may be that such criticisms invalidate a great deal of work. In any case, monozygous twins reared apart from birth are rare, it is unusual to find them known to have been dichoroidal, and it is difficult to find co-operative pairs to work with, particularly in psychosomatic or psychiatric areas. Such an approach leads to progressively smaller amounts of material and our experience has been that cooperation of the twin pairs remaining available for intensive study depends upon the patients' getting something out of the relationship.

Three cases of comparatively well studied psychosomatic disorders, investigated by teams which included medical and psychological participants, will point up features of twin investigation which have been insufficiently exploited, and will call attention to problems of evaluating twin case reports. These studies have been careful examinations of discordant and dissimilar twins, even though the most ideal criteria for selection could not be met.

CASE I. HYPERTENSION

This set of twins was first studied by Flynn, Kennedy, and Wolf, and reported in 1948 as a case of essential hypertension occurring in one of identical twins.[a] Their mother had been hypertensive for 30 years. Elsa, the twin later hypertensive, weighed 2½ pounds at birth, her sister Karen weighed 7 pounds. During infancy and childhood Elsa cried more and suffered a series of medical illnesses; at 10 months left lower lobe pneumonia and empyema required thoracotomy, and at 3 years laryngeal diphtheria required repeated intubations which were followed by chronic hoarseness. Elsa implicated this severe hoarseness as a source of her later social inhibition and recalls shyness and frequent anxiety from early childhood. Karen early became the leader and flourished in all respects but one: she frequently lost her temper. Although both were good students, she was always the better. At age 13 the girls separated; Elsa went to a trade school and became a seamstress, Karen went to a conventional high school and became a secretary. On physical examination on entering high school Elsa was found to have a systolic blood pressure of 180 mm. of mercury, but was asymptomatic. Four years later, at graduation from high school, Elsa developed transient symptoms suggestive of hyperthyroidism which were not supported by laboratory studies. Three years later, in 1948, her mother suffered hemiparesis and Elsa's recurrent headaches led to investigation at the New York Hospital, presented in detail in the early paper. Elsa's mother recovered and Elsa again became symptom-free.

At that time support for the role of anger in hypertension seemed demonstrated. The central hypothesis of the authors involved personality differences between hypertensive patients and normals: hypertensive patients were said to be nicer and more compliant but not as elastic and pliable as other people. Their main difficulties were supposed to center around the handling of rage. Hypertensives are chronically angry, unable to recognize their anger and become openly aggressive. A corollary of this hypothesis is that hypertension represents an exaggerated physiological concomitant of rage particularly in susceptible patients. A second corollary is that blood pressure increases in response to threatening situations especially in those that are likely to call forth angry feelings. Elsa and Karen appeared to fulfill the predictions of these hypotheses.

Their mother suffered a second attack of hemiparesis and coma in the spring of 1957, and it was then that Karen's hyperthyroidism was diagnosed. She was continually anxious and lost 20 pounds in weight; a PBI confirmed the diagnosis of hyperthyroidism with a level of 8.9 and she was started on Tapezole.

In September 1957, the twins were admitted to the New Haven Hospital. Karen was a pleasant, co-operative young woman. Her skin was warm and dry. There was a very fine tremor to the outstretched hand. She had a slight stare, but blinked with normal frequency. Extra-ocular movements were normal. There was no lid-lag. Lungs were clear. Her heart was small. P2 was louder than A2. There was a grade I pulmonic systolic murmur. No other murmurs were heard. The thyroid was enlarged about one and a half times normal size, a bit more on the right than on the left; no nodules were palpable. The remainder of the physical examination was within normal limits. X-ray of the chest and heart was unremarkable. A cold-pressor test showed a baseline blood pressure of 110/80; and following a two-minute immersion of her hand in ice water, her blood pressure rose only to 120/94, returning within two minutes to baseline levels of 112/86. Her blood pressure dropped off during sleep to a low at 4:00 a.m. of 90/60 which did not change significantly on her arising in the morning. Blood pepsin was 330 units; NPN was 22 mgm.%; a BEI was 4.2 gammas per cent.

Elsa was a reserved, but not embarrassed young woman. She spoke in a somewhat hoarse voice. There was a gross tremor to her outstretched hands. Extra-ocular movements were normal. A scar of a left pleural drainage was evident and there was diminution of breath sounds at the left base. Her thyroid was palpable bilaterally about one and a half times normal size. Her heart was enlarged to the sixth intercostal space, almost to the anterior axillary line. Rhythm was regular. P2 was louder than A2. There was a grade III presystolic apical murmur, a grade III rumbling diastolic murmur, and a grade III systolic murmur at the apex. At the base a grade III aortic diastolic blow, as well as a grade II aortic systolic murmur, were present. The remainder of the physical examination was within normal limits. A chest film showed left auricular enlargement with some right ventricular enlargement, prominent pulmonary vascular markings, and fluid in the left costophrenic angle. A cold-pressor test showed a baseline blood pressure of 108/80 with a rise to 146/98 after immersion of her hand in ice water for two minutes; her blood pressure returned to 106/80 two minutes after the hand was withdrawn from the water. During sleep her blood pressure fell to 130/70, but rose to 160/70 when she wakened. NPN was 28, blood pepsin was 320 units. A BEI was 5.0 gammas per cent.

Thus, it became evident that while Karen had a documented history of hyperthyroidism at this time at least, Elsa had normal thyroid function, despite the suspicion of hyperthyroidism in the past. Elsa, however, had now developed obvious rheumatic heart disease with mitral stenosis and physiologically significant aortic insufficiency. Some evidence that this might have been the case was present in the original case report where a "faint diastolic blow" was heard along the left sternal border; at the present time at least, it becomes apparent that hypertension in Elsa is much more likely to be the result of her aortic valvular lesion than it is of essential hypertension. However, her response to cold pressor and sleep tests are suggestive of a hyperactive cardiovascular response. The approach to the study was excellent and the case is historically important, yet it cannot be considered a "test case" of essential hypertension. It emphasizes the need for careful co-operation between the psychiatric and medical investigators.

Follow-up. It is interesting that the nonhypertensive, more independent twin has recently developed hyperthyroidism under circumstances that would support some of the specificity hypotheses associated with that illness. A few details will be presented. Elsa went to night school, became a secretary herself and took a job in the clothing industry, "by chance" a block from Karen. Neither has married. Karen continues to be the dominant twin, leading them into various "broadening" cultural pursuits.

Elsa was quick to agree to a follow-up study; Karen, however, didn't want any part of it at first, but was eventually won over and quickly became the active participant doing most of the talking, writing, and making decisions about arrangements. She had changed her mind because a few months before she had started to be sleepless, restless, and easily upset. This followed the second episode of coma and hemiparesis of the mother and at this time her hyperthyroidism was diagnosed. Subsequent

blood iodine studies have revealed both twins to be within normal limits while Karen was on medication.

An attractive question here involves the choice of illness. Was Karen's hyperthyroidism a reaction to the stress of a threat similar to that which had previously evoked hypertensive symptoms in Elsa? Such an hypothesis sheds no special light on stress specificity. However, the difference in character organization, with Elsa possessing hypertensive characteristics and Karen hyperthyroid ones, supports the observation that personality differences are intimately, if not casually, related to the choice of illness.

Some of the differences in the twins are obvious. Karen is more impulsive, more easily upset, more likely to get mad and "blow up." Elsa rarely experiences this kind of feeling and the family, chiefly Karen, almost automatically tends to impose on her under most circumstances.

Karen dominates Elsa to an extraordinary extent. Even in the testing and examining situation of the study, Karen not only took over the role of making all the arrangements, she also tried to dominate the study by being protective, motherly, more interested, more warm and engaging than Elsa. It was as if in the last ten years Karen had taken over the responsibility of what was going to happen to both of them. Elsa seems to have accepted this as her fate and to be resigned to it.

This speculation is supported by what seems to have happened at the time their mother had the second episode of hemiparesis. Elsa, who was concerned, but not severely upset, developed no symptoms. Ten years before, she had developed headaches when her mother was ill: then, she and her sister were still actively competing with each other. Since then, Elsa seems to have consolidated her passive role in relation to her sister and has changed her job and her way of life in order to become a closer companion to her sister. Karen, on the other hand, has also given up competing with Elsa, at the cost of assuming responsibility for her. Both girls still live at home and the ties with the parents are not yet broken, but it would seem that the loss of the mother now would be more of a blow to Karen than to Elsa.

Elsa's fantasy life appears to be richer and more troubled. Her ability to enjoy her inner experiences is relatively blocked by anxiety and by anxiety-induced restriction of functioning. Karen has a more controlled inner life and uses repression more successfully. On the surface, Karen appears to function more smoothly, independently, and vivaciously, her responses on the psychological tests have a wholesome juvenile quality, while Elsa's have the quality of an adult neurotic patient. The intellectual inhibition is striking. Elsa obtained a verbal I.Q. of 113 compared to 120

for Karen, a performance I.Q. of 121 compared to 133, and a total I.Q. of 117 compared to 128.

Karen seems to fill the clinical descriptions of hyperthyroid patients. Such patients have lively, active, and genuinely appealing qualities. Early achievement of apparent maturity, self-sufficiency, and protectiveness of others is prominent. A strong sense of responsibility founded as a defense against inner dependency and fear of death is also typical.[4,5]

CASE II. DISSIMILAR ASTHMA IN MONOZYGOUS TWINS

Crede reported asthma of markedly differing severity in identical 26-year-old men.[3] There were evident personality differences, which included separate ways of regarding the parents. The better adjusted twin, "healthier" except for his asthma, a kind, passive, pleasant person, was preferred by the parents and seemed to be making a continuous effort to please them. His asthma was much worse than that of his brother and was improved only during separation from the family while he was in the army. He was less able to be emotional, to cry or to complain, or to vent hostility than his brother. B had these characteristics to such a degree that people actually disliked him. As a consequence of this, B's adjustment in all spheres was considerably poorer than A's, but he had minimal asthma.

Independent psychological testing supported the clinical appraisal in this pair. A follow-up recently obtained from Dr. Crede reveals that twin A, after psychotherapy, now also has minimal asthmatic difficulty, but that B probably has had a brief psychotic episode and continues to have considerable social difficulty.

The special importance of this case, well recognized by the original investigators is the necessity of fitting case material into an holistic frame; that is, one should not be deceived into seeing differences between twins simply as a matter of totally "better" or "worse" background situations.

CASE III. DUODENAL ULCER IN ONE OF IDENTICAL TWINS

A case of peptic ulcer in one of identical twins, has been published;[2] here the physiological parameters such as blood pepsin and gastric intubation studies were the same, but differences in character structure and the degree of external distress were quite evident. The subjects are strikingly similar in background and appearance. They make the same initial impression; passive, shy, dependent, anxious, semi-skilled workers. The ulcer twin began to have symptoms during a near-psychotic breakdown in his wife when she was having an affair with another man and threatened to kill their children. The patient's difficulty with his wife, though somewhat diminished, has persisted since the onset of illness and so have the ulcer symptoms. In contrast, his brother is married to a motherly woman who dependably manages family affairs. Both patients have high blood pepsin levels, but show on interview and psychological testing, modest but real differences in their responses to stressful circumstances. Although superficially similar, the ulcer twin is more shut off, resentful, bitter, and less capable of having satisfying relationships. Blind analysis of projective test results highlighted certain personality differences and correctly identified the ulcer twin.

This case study supports the hypothesis that the level of blood pepsin is genetically determined and that the occurrence of ulcer depends upon the presence of gastric hyper-

secretion, plus a special combination of circumstances. We found support for Alexander's conception of a type of passive-dependent personality who develops peptic ulcer in the presence of circumstances which threaten the nurturance of these needs.

DISCUSSION

Of considerable value from the dynamic point of view is the study by Kent of 19 pairs of twins seen in a child guidance agency.[7] Her findings suggest that the superficial environment of the family may appear to be remarkably similar for each one of the twin pair: there is no separation of the twins, the family is intact, the twins are physically identical, go to the same school, wear the same clothes, and yet these are coarse similarities. Individual twins may live in a markedly different intra-familial environment; the different concept of the parents about each twin (for example, identification of separate twins with other family members by the parents), and the division of roles between the twins, especially as regards dominance, competitiveness, and leadership, form two obvious bases for such differences.

The study of illness in monozygous twins offers more than information about the genetic determinants of disease. The shaping of development, normal and pathological, is illuminated by such studies. For the development of any character trait, symptom, or illness, many factors are usually involved; there are now hundreds of physiological and psychological studies related to developmental shifts and changes. Careful study of single cases or small groups of cases with physiological and psychological measurements can be used to test hypotheses concerning various factors in illness.

Certain pertinent issues deserve further comment. The problem of susceptibility to illness and the identity of the physiological response in twins are relatively unexplored areas. Physiological studies have shown close similarities within twin pairs, with respect to onset of puberty and level of blood pressure, gastric secretion, blood pepsin, electrocardiogram, and electroencephalogram, and point in the direction of identical patterns. In general, basic physiological processes are more narrowly defined in monozygous twins. The problems of susceptibility to illness based on integrated psychosomatic concepts is much more difficult to answer as it depends in part on the development of differences including personality de-differentiation. While there is a natural tendency for monozygous twins to develop along similar lines, a tendency reinforced in part by identification with each other and in part by a lack of distinction between the twins by the parents, other factors tend to result in personality differences.

Through careful accumulation of discordant and dissimilar twin cases, it may be possible to check the relevance of infantile development, character structure, and environmental stress in the development of illness. "Psychosomatic" illness offers an especially intriguing laboratory for such investigation because of the availability of measurable physiological data.

The three cases presented do not by themselves solve the central problem of character predisposition vs. "stress" as the source of triggering mechanisms in disease. Knowledge that an illness has genetic determinants does not reduce the importance of looking for other factors that may play a role. If an intimate relation between a general line of development and an illness exists in one of identical twins, it must still be noted that the original source difference can be intrauterine, but not genetic.

Still, even this criticism of discordant cases may not be well founded; if it can be established that the twins studied are not greatly different from other twin pairs except in the one pattern of development under scrutiny, if this pattern can be shown to be crucial for the symptom, character trait, or illness which develops, such a study of twin cases will continue to be of value in determining the genesis of disease.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Roy Schafer, Ph.D., for his evaluation of the psychological materials.

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EVENTS

WOODWARD LECTURE

September 25, 1958

DIGEST OF LECTURE ON HOME CARE. By John Scott, Medical Director, London County Council, England.

Doctor Scott dealt with the home care of the sick, particularly the elderly sick in London, and added some details of the tuberculosis situation. The key figure in home care was the general practitioner, with whom about 98 per cent of the population were registered and who received an annual capitation fee for each person on his list. The London County Council area had a population of 3½ million, the expectation of life at birth being 68 years for men and 72 for women. In 1901 the proportion of the population over 65 was 1 in 21, now it was 1 in 9 and by 1975 it would be 1 in 7. This raised problems of retirement, of suitable housing, and possibly most important, of loneliness, since in England and Wales as a whole more than one million elderly people lived alone. To help ease this feature there were in London alone 30 voluntary committees which provided clubs, recreations, handicrafts, and meals at cheap rates. In addition, for the housebound, meals were delivered to the home twice weekly. For the sick, there was a separate subsidized voluntary service delivering week-day meals five times weekly, about 180,000 such meals being supplied annually.

Assisting the general practitioner in his care of the sick and the elderly were three important services supplied by the health department: the public health nurse, the visiting nurse, and the homemaker. The public health nurse was in touch with all other official and voluntary agencies and could often see that various needs of the elderly were met. The visiting nurse was again an invaluable auxilliary of the general practitioner. London had 550, with an average case-load of 24; they made 12-14 home visits daily and were nursing an average of 13,000 cases at any one time. They completed some 63,000 treatments each year, nearly half their patients being over the age of 65. Cases of respiratory disease made a quarter of the total, diseases of the heart and arteries (14 per cent), skin 19 per cent, digestive diseases being the next most frequent groups. Each year these nurses paid about 1,400 visits per 10,000 population. Approximately 80 per cent of their calls came from general practitioners and 17 per cent from hospitals. Apart from a first emergency visit if necessary, their work was done entirely under medical direction and, as part of the National Health Service, was free.

The homemaker service was just as valuable and in London employed the equivalent of over 2,000 full-time workers. Thirty-five thousand cases were assisted each year, of whom 80 per cent came under the category of aged and chronic sick. If necessary, they attended in the early morning or late at night. This service was charged for in accordance with means, but less than 10 per cent of the cost was recovered. About 40 supervisors ensured that the service was carried on economically as well as efficiently; the average time per household per week in London was under 4½ hours.

It made a great contribution to keeping the old and the sick in the homes to which they were attached.

The speaker also touched briefly on housing provision for the aged, on the welfare homes which accommodated nearly 9,000 in London, and on mental health problems in old age. Dr. Scott then turned to some aspects of tuberculosis in London. In 1905 it caused 12 per cent of all deaths, today only 2 per cent. The incidence, as shown by notification figures, had dropped less dramatically, being still about 1.1 per 1,000, deaths being 0.16 per 1,000. A quarter of a million people were mass x-rayed each year, uncovering about 1,300 cases of active tuberculosis. In London, as elsewhere, the picture in men had changed dramatically: 87 per cent of male deaths from the disease occurred in men over 45. About 4,000 child contacts were vaccinated with B.C.G. each year and a national programme also offered routine Mantoux testing to 13-year-olds, with B.C.G. vaccination if necessary. In London there were about 30,000 children each year in this age group, of whom 86 per cent were Mantoux negative. Since 1954 about 20,000 children annually had been vaccinated, and it was confidently believed, given valuable protection.

IRA V. HISCOCK

October 9, 1958

EXPERIMENTAL STUDIES ON THE MECHANICS AND PHYSIOLOGY OF NOCTURNAL MIGRATION IN BIRDS. By Franz Sauer, University of Freiburg, Germany.

Experiments with four species of European warblers have revealed that nocturnal migrants can orient by means of the stars. Ringing returns show that western European populations of Blackcaps, Garden Warblers, and Whitethroats travel SW to South Africa on fall migration and that Lesser Whitethroats travel SE. When the last species reaches the eastern end of the Mediterranean, it changes its heading to S, ending its migration in central east Africa.

Birds were placed in a cage surrounded by black felt to block extraneous clues and exposed only to a 68-degree circular section of the night sky. Movements of the birds fluttering on a rotatable ring perch inside the cage were observed from below. Nonrandom patterns of fluttering direction were recorded during migratory seasons.

When exposed to the starry sky on clear nights, birds showed identical fluttering directions with those normally assumed on migration. This was true for both wild-captured adults and brooder-raised young birds which had never seen a starry sky. If the ring was rotated, the bird turned accordingly to readjust to the preferred direction. Birds isolated from hatching which had never experienced a starry sky fluttered at random for three minutes and then behaved like wild-caught members of the species. Moonlight and shooting stars exercised a positive phototatic influence on the experimental birds; cloudy conditions gave uncertain patterns; and in overcast conditions, birds were completely disoriented. Complete dis-

orientation was also shown in experiments conducted indoors with diffuse and polarized light.

Lesser Whitethroats placed in the cage under an artificial sky of a six-meter planetarium at Bremen responded similarly to those watched under natural starry skies. Under star patterns corresponding to these at 50, 45, and 40 degrees north, the birds oriented SE. If the planetarium sky was shifted to an apparently more southern aspect, the bird oriented SE and S at 25 and 20 degrees and S at 15 and 10 degrees. Brooder-raised isolated birds without previous migratory experience showed the same behavior pattern as the species apparently does on its way to Africa. Under a high latitude sky, the birds are in tension, but when sky conditions are shifted to 15 or 20 degrees North, birds are weakly motivated.

A planetarium eastward shift to a Siberian sky was presented to a Lesser Whitethroat. The bird could react in one of three ways depending on its interpretation of what the sky revealed. The star pattern was visible at the precise time presented 70° 30′ to the east of the Bremen planetarium; 5 hours 20 minutes later it would be seen over the experimental planetarium; and 77 days later in the bird's natural winter quarters. If the bird felt itself displaced to the east, it should head west; if it adjusted its internal clock 5 hours 20 minutes later, it should head SE; and if it adjusted the clock 77 days it should cease migratory behavior altogether. The bird responded as if moved to the east. It headed NW and W in the cage until shown a sky apparently near the Carpathians, when it gradually veered SW and S. The internal clock, therefore, did not adjust.

If the bird was "taken" west, it headed E; but if "returned" east and again "taken" west it headed SE. A summer or December sky shown to a bird in the fall migratory period caused disorientation; but if the bird was again shown a fall sky, it immediately oriented SE. Therefore, the orientation pattern is precise in time.

Preliminary experiments with sky fractionation have revealed that the well-defined Milky Way is not important for orientation; that the southern half of a sky is important to a bird orienting S; that when 1/32 of a southeast sky is blacked out, a SE heading bird faces NW, but that if 14 to 18 stars are returned to the blacked-out area, the bird re-orients SE. Although some quadrants of the sky appear to be important, exactly what star patterns are significant for orientation has not yet been shown.

G. E. W. 3RD

YALE MEDICAL SOCIETY

October 14, 1958

MECHANISMS OF ACTION OF CORTISONE. By Dr. C. N. H. Long, Sterling Professor of Physiology, Yale University School of Medicine.

Although cortisone, like all the cortical hormones, influences salt and water balance as well as intermediate metabolism, the present discussion will be limited to its "organic" effects.

After adrenalectomy, the fasted animal develops hypoglycemia, with a reduction of gluconeogenesis as reflected by a decreased nonprotein nitrogen in the urine. These animals, if given hydrocortisone subcutaneously, show a sharp rise in blood glucose, which reaches a plateau in five hours, a slow rise in liver glycogen, and a still slower rise in muscle glycogen. In *fasted* animals the hyperglycemic effect of administration of the 11-oxysteroid seems to be due to the stimulation of gluconeogenesis through protein catabolism. Surprisingly, although adrenalectomized *fed* animals show ostensibly the same hyperglycemia upon institution of hormone therapy, the effect of cortisone here is on carbohydrate utilization and glycogenesis. A decrease in glucose oxidation and an increase in glycogenesis precede by greater than three hours any changes in protein breakdown. It has also been observed that the administration of hydrocortisone for five days causes a steroid diabetes which differs from the pancreatic type in that hyperglycemia is the result of the overflow of glucose after saturation of carbohydrate stores and can be reversed upon removal of the hormone. Adrenalectomy can ameliorate diabetes mellitus of pancreatic origin.

The liver figures prominently in the action of adrenal steroids, as indicated by the inability of animals to form hepatic glycogen in the absence of the hormone and by the ineffectiveness of the hormones in regulating carbohydrate breakdown in the absence of the liver. Winternitz has shown that the loss of muscle glycogen following the injection of epinephrine greatly exceeds the sum of the increases in liver glycogen and blood glucose, in adrenalectomized rats. If given glucose or fructose, the corticectomized animal shows a reduced ability to synthesize liver glycogen. The defect does not lie in the inability to form glucose from precursors. An intriguing possibility to explain the role of the liver is that cortisone normally acts to inhibit the hexose monophosphate pathway, in which glucose is oxidized through pentose intermediates. Nucleotide synthesis, involving pentoses, may be reduced after injection of cortisone and the glucose diverted to glycogen formation, with the subsequent appearance of a negative nitrogen balance.

M. S. M.

ZOOLOGICAL JOURNAL CLUB

October 17, 1958

THE CHEMICAL GENETICS OF HEMOGLOBIN. By Harvey Itano, National Institutes of Health, Bethesda, Maryland.

Interest in the sickle-cell phenomenon has led to a physicochemical study of the properties of hemoglobins A,S, and C, and has provided information that bears on the mode of gene action of the three alleles associated with these molecular species.

Hemoglobin molecules are symmetrical dimers, each half-molecule being composed of two dissimilar peptide chains and two heme groups. In acid solution dissociation into two sub-units takes place; reconstitution occurs at neutral pH. When mixtures of A and S are so treated, mixed dimers

cannot be detected electrophoretically. Conversely, in reassociated mixtures of methemoglobin C with either S or A, new molecular types are found. This fact can be explained by assuming asymmetric splitting of the original dimer so that two pairs of like peptides remain together, i.e., in S, the two quarter-molecules containing valine in place of glutamic acid remain together. Work with S. J. Singer and using C^{14} marked hemoglobins and starch gel electrophoresis of reassociated molecules supports this conclusion completely.

This information must cause re-examination of the hypothesis that A, S, or C loci are responsible for the production of half hemoglobin molecules, each composed of two unlike peptides (the dimer being two such units). The asymmetric splitting data would mean that dissimilar peptides are separated from each other more easily than from their duplicates in the whole hemoglobin molecule. This suggests the possibility that two loci may be involved in hemoglobin production, each being related to the production of a different peptide type. The dimer then might arise when double units of dissimilar peptides associate, as they did in these experiments. Until further genetic evidence is obtained, no choice can be made between the hypotheses.

N. K. WESSELLS

BOTANY SEMINAR

October 21, 1958

BENEFICIAL AND HARMFUL EFFECTS OF PLANT VIRUSES ON ANIMALS. By Karl Maramorosch, Rockefeller Institute for Medical Research, New York City.

The aster leaf-hopper *Macrosteles divisus* is both the vector and alternate host for the aster yellows virus, a disease affecting asters and many other plants. It has been shown that the virus both moves and multiplies in the insect and is found in the blood. The virus is transmitted to a new host plant via the insect's saliva. After a long period of infection the fat body of the male *Macrosteles* is pathologically altered, with stellation of cell nuclei and loss of granular cytoplasm. No other deleterious effects are known at present.

Another leaf-hopper, the semi-tropical *Dalbulus maidis,* is normally able to feed and survive only on the corn plant. It will attempt to feed on asters, but will soon starve; during the feeding attempt it may also become infected with aster yellows virus. The virus is never found in the saliva of this insect, which thus cannot act as a vector. Once *Dalbulus* is infected, however, it was found that it *can* feed and survive on either infected *or healthy* aster plants. But corn is still necessary for reproduction or for molting of nymphs. Exposure to a temperature of 36° C. kills the virus; infected insects that are so treated are no longer able to feed on healthy asters, presumably due to absence of the virus.

It is believed that the virus, acting directly on its animal host, is responsible for the increased survival of aster-fed *Dalbulus.*

F. M. W.

BIOPHYSICS SEMINAR

October 23, 1958

THE MECHANISM OF VIRUS-ANTIBODY UNION. By S. Fazedas de St. Groth, Australian National University.

The difficulties in studying the virus-antibody reaction are well illustrated by considering logarithmic plots of the number of viruses neutralized versus the titer of antibody under experimental conditions using the chick allantois or amnion, and the mouse lung as host. Contrary to expectations, curves with different slope and shape are observed.

In order to overcome such difficulties a mathematical treatment of the virus-antibody reaction is put forward. The equilibrium constant (K) of such a reaction may be expressed $K = \frac{(sV\text{-}x)\ (A\text{-}x)}{(x)}$, where V = the number of viruses, s = the number of antigenic sites per virus, A = the number of antibody particles, and x = the number of antibody particles combined with viruses. The number of surviving viruses (Vs) is then $Vs = V\ (1\text{-}p)^c$, where p = the chance of any one site on a virus being occupied, and c = the number of sites that can neutralize a virus. If p is estimated as $\frac{x}{sV}$, then $Vs = V\left(1 - \frac{A}{nsV+A+K}\right)^c$, where n = the number of viruses that are antigenic but not infective. Furthermore, if $nsV << K$ and $\frac{A}{K} << 1$ hold, then $\frac{V}{Vs} = \left(1 + \frac{A}{K}\right)^c$, or $\log\log\frac{V}{Vs} = \log A + (\log c - \log K)$. Plots of this equation yield a straight line with unit slope; the intercept with the abscissa being referred to as the "activity," or pN, a term analogous to pH.

Experimental data of the influenza virus permits defining the variables; thus $K = 10^{13}$ to 10^{15}, s = 2700 sites per virus, and c = 9.5 sites. Data of the yellow fever and vaccinia viruses also fit the equations and lend evidence that the assumptions made in the derivation were valid. Therefore, the equations may be used in comparing antisera and in predicting how virus and antibody will act *in vivo*.

N. K. WESSELLS

October 27, 1958

STUDIES OF NOCTURNAL MIGRATION BY RADAR. By Ernest Sutter, University of Basel, Switzerland.

In order to follow the course of bird migration during the night when direct observation is impossible (and also under adverse daytime conditions), use was made of the radar facilities of the Zurich airport. Small passerines are detected only if flying in flocks; thus the radar picture is not complicated by the random flights of solitary, nonmigratory birds. The studies were made during the autumn, when a great number of birds migrate through this part of Switzerland, most in a southwest direction.

Typically, migration is minimal during the daytime, increasing sharply at about 6:00 p.m., rising to a maximum about midnight, and then decreasing slowly until morning. Weather conditions can radically alter the number of migrants, as well as their direction and altitude of flight.

Migration is maximal under conditions of clear weather and decreases with increasing overcast. Thus, birds migrate under just those conditions which enable them to see the stars, which Sauer has shown to be used for navigation. On a rainy night the birds aggregate over Zurich; in the absence of stars they are drawn to the city lights. In a fog, two strikingly different components are seen, one flying in the normal southwest direction and the other flying about in a seemingly random manner. It is believed that the oriented birds are above the fog and the disoriented ones below it; the former see the stars and the latter do not.

Wind velocity and direction also have a great effect. The number of birds is greatest during calm or northeast, i.e., tail, wind. Usually an increase in wind velocity is accompanied by a decrease in number of migrants. Those that do migrate at wind velocities above 15 to 20 knots tend to be carried in the direction of the prevailing wind.

In optimal weather, the radar shows as many as twelve hundred flocks crossing a one mile front per hour; each flock is guessed to consist of about ten birds on the average. Thus, there are many thousands of birds passing each night.

F. M. W.

October 29, 1958

DIFFUSIBLE MOLECULES IN CELLULAR DIFFERENTIATION. By Charles E. Wilde, Jr., University of Pennsylvania, Philadelphia, Pennsylvania.

Various aspects of the differentiation of a Metazoan organism are believed dependent upon molecular interactions of dissimilar tissue types. That such interactions may sometimes occur via diffusible molecules is suggested by experiments relating phenylalanine metabolism to the differentiation of neural crest pigment cells in the salamander *Amblystoma maculatum*.

None of the cells derived from dissociation of a mass of neural crest (stages 10.5, 11), and grown *in vitro*, differentiate into melanin containing pigment cells unless 1.0mM/100ml. of phenylalanine is present in the medium. Possible precursor molecules for the amino acid, such as phenylserine or phenyl-lactic acid, have no sparing effect, i.e., no melanin is formed. Conversely, addition of cells from the archenteron roof mesoderm to such a culture system results in pigment formation in competent neural crest cells; the implication is that mesodermal tissue can use the precursor molecules in such a way that neural crest cells growing in the same vessel can complete their differentiation.

A similar ecto-mesodermal relationship exists in the ventral embryonic region. Ventral skin cells that never normally become pigmented *in vivo* do so when treated with from 2.0 to 8.0mM/100 ml. of phenylalanine *in vitro*. Furthermore, while such ectoderm will not form melanin in the

presence of the precursor molecules alone, it will do so when cells from the subjacent mesoderm are added to the culture medium.

That the ability to form melanin resides mainly in the cytoplasm was demonstrated by growing anucleate pieces of neural crest cells (obtained by centrifugation through fiber-glass) in the presence of phenylalanine. Melanin granules appeared in the "pure cytoplasmic" masses.

The intimate relation of phenylalanine and its precursors to the differentiation process in the neural crest-archenteron roof system suggests that such a diffusible molecule may be associated in a major way with at least one phase of the induction phenomenon, and further, that analogous systems may exist elsewhere in developing embryos.

N. K. WESSELLS

WOODWARD LECTURE

November 6, 1958

THE FEATHER. By Erwin Stresemann, University of Berlin, Germany.

The most complicated integumentary structure of the vertebrates is the Avian feather. Gross and microscopic anatomical studies have revealed a multitude of relationships between feathers and various aspects of bird life.

The precise adaption of feather morphology to function is revealed by studies on the buoyancy of ducks. The arrangement of the barbs on the contour feathers, and of the feathers themselves, is such that water cannot pass between the closely placed structures and thereby "wet" the duck. Buoyancy results from this phenomenon, not as a result of an oily secretion coating the feathers as has long been believed. In recent experiments oil glands were removed from ducks; after several weeks the feathers were carefully cleaned and the ducks placed in water—they swam perfectly. When a detergent (that lowered the surface tension) was added to the water, however, buoyancy was reduced and the ducks sank so that only their heads remained above water. This result demonstrates the precision that is characteristic of the adaptive mechanism, for the feather barbs function normally in keeping the duck afloat only in water with normal surface tension—there is no means of altering the morphological pattern in immediate response to a lowering of the tension.

Thus, besides being prerequisites for flight, and being the site of ecologically and ethologically important color, feathers also are the source of buoyancy for at least some aquatic birds.

N. K. WESSELLS

BOOK REVIEWS

THE PHYSIOLOGY AND PATHOLOGY OF THE CEREBELLUM. By Robert Stone Dow and Giuseppe Moruzzi. Minneapolis, The University of Minnesota Press, 1958. 675 pp. $12.50.

In 1673 DuVerny reported that he had been able to maintain pigeons in which both cerebellum and cerebrum had been extirpated. From such almost parenthetical beginnings this volume by Drs. Dow and Moruzzi traces, with scholarly thoroughness and clarity, the evolution of our present knowledge of the cerebellum.

Because of the complexity of cerebellar function, a wide variety of investigative methods have been applied through the years to the study of this structure. The authors have utilized this fact to present an orderly summary of information which is copious and often controversial.

The volume consists of two major sections: the first, comprising two-thirds of the book, covers the physiology, the second, the pathology and clinical aspects of the cerebellum. The physiological portion of this work is presented in terms of the various experimental eras through which cerebellar investigations have passed. It begins, after a brief historical introduction, with the ablation experiments, then proceeds through the stimulation and electrophysiological experimental stages to the present. In three final chapters in the first portion of the book the relations between the cerebellum and other central structures, developmental physiology, and general functional considerations are discussed. The mass of research data presented is voluminous, and the difficulty of the task compounded by the many contradictory results, by the lack of comparative physiological study in this field, and, finally, by the still nondefinitive status of much of our current knowledge. Despite these obstacles, the authors have presented a finely formulated and coherent picture of their topic. Particularly valuable are the discussions of Luciani's atonia, fastigial function, and the influence of the cerebellum on gamma discharges.

The second section of the book is also comprehensive and notable for the correlation of clinical with pathophysiological data. It is probable, however, that the greatest value of this volume will prove to be its exhaustive summary of physiological research. The entire volume is richly supplied with references.

In keeping with the highly academic tenor of this study, the authors have included numerous quotes from a variety of investigators—and in a variety of languages. In some instances these occur at vital points of the text. A glossary of translations of these quotes might prove a worthwhile adjunct to future editions.

This work presumes a thorough knowledge of cerebellar anatomy and is, in fact, a companion piece to Professor Olof Larsell's as yet unpublished text on this subject. This excellent volume by Drs. Dow and Moruzzi, representing the most inclusive work on cerebellar function to date, will be invaluable to both researchers and clinicians in the neurological field. One may well look forward to its forthcoming anatomical counterpart.

DONALD W. COOPER

PHYSICAL METHODS IN PHYSIOLOGY. By W. T. Catton. New York, Philosophical Library, 1957. xi, 375 pp. $10.00.

This little book contains a surprisingly large number of good descriptions of experimental techniques, presented with enough theory to make them intelligible. The chapters deal with blood, the circulation, respiration, muscle, peripheral nerve, the physical basis of body heat exchanges, and electronics in physiology. The methods are all physical rather than chemical, and such topics as electro-cardiography, electro-encephalography, and the special senses have been intentionally excluded. Among the many instruments described are an improved photoelectric oximeter, an electromagnetic flowmeter for blood in an artery, an infra-red analyzer for carbon dioxide gas, and a transistor stimulator circuit. The 290 references should make the book especially useful to students planning to begin physiological research.

DAVID I. HITCHCOCK

GENERAL ZOOLOGY. By Claude A. Villee, Warren F. Walker, Jr. and Frederick E. Smith. Philadelphia, W. B. Saunders Co., 1958. xix, 877 pp. $7.50. LABORATORY EXERCISES IN GENERAL ZOOLOGY. By Harvey I. Fisher and James B. Kitzmiller. Philadelphia, W. B. Saunders Co., 1958. v, 227 pp.

General Zoology appears to be a remarkably complete and up-to-date college text. It is complete both in the wide range of topics covered and in the detail with which they are covered. It is up-to-date not only in the sense of containing much new information, but also in the sense of following the trend of the times; a great deal of emphasis and space are devoted to dynamic and physiological principles. For example, a full chapter each is devoted to endocrinology and to arthropod physiology and behavior. Such emphasis is not without its toll on the more descriptive material of the book, but adequate coverage (if not balance) is nevertheless maintained. The text is divided into five main sections.

The book begins in a relatively sophisticated manner with a section on "General Concepts"—a discussion of the concepts of cell, tissue, metabolism, physiology, and reproduction. There is much here that will befuddle, at least temporarily, the freshman who has had inadequate training in chemistry (although the authors do explain many of the concepts as they are introduced), but he will nonetheless be all the better off for learning at the outset that this is one very important way of thinking about biological phenomena.

"The Animal Kingdom" is a survey of the invertebrate phyla, covering for each not only taxonomy, anatomy, and natural history, but also many basic biological concepts as they best fit a particular group. For example, there are discussions of regeneration in Turbellaria; reproductive periodicities in Annelida; cell constancy and aging in Rotifera; and neurosecretion, orientation, and social behavior in Arthropoda.

"Vertebrate Life and Organization" begins with a detailed description of the traditional "representative vertebrate" (frog), followed by three chapters on the evolution and characters of the vertebrate groups, six chapters on organs and their physiology, and one on embryology. The interplay of structure and function presented in the last seven chapters, especially the

one on endocrinology, should provide the student with an excellent over-all view of the integration of organic systems.

"Genetics and Evolution" contains a reasonably clear and detailed discussion of the principles and problems in these fields, including gene action, the origin of life, and a good summary of the evidence for evolution. Some may feel that this section should come before that on vertebrate evolution; the anatomical detail in this section is not so great as to prohibit such an inversion (perhaps it should be read both before and after).

"Animals and their Environment" contains the principles of syn- and autecology, adaptations to various environments, parasitism, and conservation. The clarity and detail of these sections suffer perhaps from lack of space, but it is on the whole adequate.

There are questions and references at the end of each chapter, and an appendix containing a concise taxonomic summary. The illustrations are in general clear, but occasionally too small for comfortable perusal (e.g. *Busycon*, Fig. 14.5, p. 249). The text is too long for complete coverage in a half-year course, but topic selection can be made without too much difficulty. For example, genetics is split into two chapters, the first giving just the bare elements of Mendelian theory needed for an attenuated course.

In general, the authors seem to have done a remarkable job of avoiding a great number of the half-truths that usually result from over-simplification. The student should thus obtain a thorough but flexible grounding in zoological principles, amenable to expansion and revision with later study. The book is thus recommended for use in introductory college courses, especially full-year courses.

Laboratory Exercises in General Zoology is apparently designed as a companion volume for the above text, and is so recommended by both its authors and publisher. It is a relatively complete, well-organized laboratory outline, with much explanatory material prefacing each exercise. It is organized in such a way that there should be little serious discrepancy between laboratory and lecture progress if the chapter order of the text is preserved. There is a minimum of living material presupposed (except for readily obtainable forms such as can be got from most localities or biological supply houses), but addition of any available living specimens is both easy and desirable. The reviewer objects to the extensive use of outline drawings to be completed and/or labelled and handed in (except, of course, outline maps for biogeographical study), for it may encourage carelessness in observation and visualization. Of course, it can be required that the student not use the exercise sheets in the manual except as a *guide* for making their own reports. On the whole, this manual is of sufficient quality to be recommended for use with both the above-reviewed and many other textbooks.

F. M. W.

MANUAL OF MICROBIOLOGICAL METHODS. By the Society of American Bacteriologists Committee on Bacteriological Technique. New York, McGraw Hill Book Company, Inc., 1957. x, 315 pp. $5.50.

This book might better be named "Selected Topics of Bacteriological Technique" for such is the subject matter, to which has been added a chapter on

virological methods and physiological and biochemical techniques. Nearly half of the subject matter of microbiology is omitted, and the reader will look in vain for any mention of laboratory methods dealing with malaria, leptospirosis, syphilis, histoplasmosis, trichinosis, the tineas, etc., etc. The bacterial viruses receive scant mention and the plant viruses and fungi none, although there is a chapter on plant bacterial pathogens.

Each chapter is written by a different author or set of authors on a particular topic with which they have had extensive experience. They are, in general, very well done and reflect expert, specialized knowledge. Most of the chapters appear to be directed at beginning students or technicians, a few discuss general principles. References accompany each chapter, five of which are new and these have the most modern bibliography. The topics are selectively, not exhaustively, treated, the techniques recommended are in general reliable in so far as the reviewer is familiar with them.

The book replaces a series of leaflets formerly issued, from time to time, by the Committee and possibly for that reason is a curious mixture of excellencies and deficiencies. The excellencies result from the familiarity of the authors with the topics discussed, the clarity of the directions, and the reliability of the methods. But there is a desultory quality about the whole work suggesting the lack of an over-all plan, a failure to agree on level of presentation, an arbitrary selection of materials to be covered, and a variable degree of modernity. It can be recommended within narrow limits and might with advantage be added to a library which already contains several books on microbiological technique with broader and more systematic coverage.

DAVID WEINMAN

TUBERCULOSIS IN WHITE AND NEGRO CHILDREN. VOLUME I, THE ROENTGENOLOGIC ASPECTS OF THE HARRIET LANE STUDY. By Janet B. Hardy. Published for the Commonwealth Fund by Harvard University Press, Cambridge, Massachusetts, 1958. 122 pp. 87 figs. $7.50.

This monograph represents the roentgenological aspects of tuberculosis in children as seen in the Special Tuberculosis Clinic at The Harriet Lane Home from 1928 to 1950. It includes the study of 1,329 children—892 Negroes and 437 whites.

The four-page introduction represents an excellent brief summary of tuberculosis terminology and definitions which is worthy of the attention of all interested in concise descriptions in tuberculosis. This is followed by a consideration of the basic anatomy of the respiratory tree distal to the larynx. A two-page consideration of the basic principles of interpretation of chest roentgenograms completes Section I of the monograph. This short discussion, although of necessity limited, is well presented.

The remainder of Section I consists of a large number of x-ray reproductions which cover the various phases of tuberculosis as seen during childhood. The case material is well chosen and is an excellent compilation from a wide clinical experience. The reproduction of the radiographs, however, is in most instances of only average quality, thereby detracting significantly from the value of this beautiful collection of x-rays.

The last section of this work describes the methods of bronchography and bronchoscopy used at the Harriet Lane Home. The emphasis on meticulous care in the performance of the anesthesia for bronchography and bronchoscopy is a welcome addition to this field. Especially important is the emphasis upon careful limitation of the total amount of anesthetic agent to be used in patients.

The monograph represents an excellent presentation of the roentgenological aspects of childhood tuberculosis and is marred only by the average reproduction of the roentgenogram.

RICHARD L. BARACH

ACTION OF RADIATION ON TISSUES. An Introduction to Radiotherapy by A. Lacassagne and G. Gricouroff. Translated by C. C. Lushbough, M.D., and Gretchen R. Riese, M.S. New York and London, Grune and Stratton, Inc., 1958. viii, 199 pp. $6.25.

This is the second edition, greatly modified and expanded in certain vital aspects since the original publication in 1941. The opening chapter discusses general concepts of the action of radiations. Following this the biological effects on each of the normal tissues of the body are described. The concluding chapters cover general manifestations of total body irradiation and the action of radiation on pathologic tissues.

Each section is developed historically, and the early experimental evidence of radiation damage to normal tissues is cited. Much of the material covers animal experimentation. Changes on a cellular level are dealt with primarily, and cellular radiophysiology is particularly stressed. Gross reactions to radiation are not discussed at length except in the chapter on total body irradiation. Extensive references are given by the authors which cover the past development of knowledge in radiobiology as well as the more recent literature. Most of the references are European in origin. The translators have supplied an additional bibliography of the more recent American contributions to the subject. A number of excellent illustrations enhance the value of the book.

As described in the subtitle, this book serves as an introduction to the clinical aspects of radiotherapy. It should be very helpful to young physicians who are just entering this field. By adding clinical experience to the fundamental knowledge included in this volume the radiotherapist will better understand the early and late changes observed in the normal tissues of patients undergoing radiation therapy.

HAROLD L. ATKINS

CLINICAL ENZYMOLOGY. Gustav J. Martin, Ed. Boston, Little, Brown & Company, 1958. 241 pp. $6.00.

Written by Dr. Martin, the Research Director of the National Drug Company, and five other contributors, this work is a brief review of the chemical and biological nature of certain enzymes, their modes of action, factors that influence their activity, and some of their diagnostic and therapeutic applications.

Of the seven chapters, the three written by the editor are the most original and challenging. "Protein Biology" describes some of the characteristics of enzymes as proteins, discusses the role of enzymes in the structure and function of living matter, and touches on biological relativity. The final two chapters by Martin are a rather delightful if eclectic mixture of technical examples and philosophical musings, the one entitled "Polymerases in Biology" being especially provocative.

The intervening chapters deal with more specific features of enzymes having some current clinical applications. One section describes the physical characteristics and general methods of isolating and defining trypsin, chymotrypsin, ribonuclease, cholinesterase, and hyaluronidase, ending with a review of enzyme kinetics. While the chapter devoted to the biochemistry of enzymes is quite thorough and informative, the following one dealing with their parenteral administration is rather tedious, uncritical, and inconclusive. Similarly, the section "Diagnostic Use of Enzymes" is clinically naïve (". . . elevated serum amylase levels are pathognomonic of acute pancreatic disorder"), a bit outdated (only brief mention of serum glutamic oxaloacetic transaminase in a cardiac-oriented fashion), and incomplete (no comment about hypophosphatasemia).

As a whole, this book is quite erratic in style, probably a result of the many contributors, and alternates between plodding tabulations of clinical trials and ethereal speculations about enzymes and Life in General. Its chief function will be to serve as background reading for students and physicians; much of it is too theoretical for technicians, the rest is too simple for biochemists.

WILLIAM A. TISDALE

GESTATION. Transactions of the Fourth Conference. Claude A. Villee, Ed. New York, Josiah Macy, Jr. Foundation, 1958. 216 pp. $4.50.

This monograph includes the proceedings of the Fourth Conference on Gestation. Four topics are discussed: The Physiology of Fetal Fluids, Jack Davies; The Origin of Amniotic Fluid, Albert A. Plentl; The Morphologic Manifestations of Uptake of Materials by the Yolk Sac of the Pregnant Rabbit, Sarah A. Luse; and The Development of Fetal Immunity, F. W. Rogers Brambell.

The Physiology of Fetal Fluids concerns itself chiefly with the differential transfer of glucose and fructose from lumen of the rabbit uterus to the amniotic fluid. The Origin of Amniotic Fluid is of more interest to the clinician, in that it again points out the very marked rapidity with which the amniotic fluid volume is exchanged. On the basis of work of the current author, as well as the others, it has been fairly well established that the amniotic fluid in the human is replaced every three hours. It is also pointed out that the water, sodium, and potassium exchanges are all apparently independent of each other. Radioactive deuterium and tritium are used in arriving at the conclusions concerning the rapidity of amniotic fluid flow.

To the reviewer the most interesting part of this article is a deep philosophical discussion of the evaluation of methodology between Doctors Fremont-Smith, Plentl, Davies, and others. This is a carefully presented article

and could be read with profit by anyone interested in the problem of hydramnios.

The Morphologic Manifestations of Uptake of Materials by the Yolk Sac of the Pregnant Rabbit describes how colloidal gold, erythrocytes, cream, and protein, in the form of egg white, are absorbed by the yolk sac. There are several electron microphotographs which are used to illustrate some of the processes.

The Development of Fetal Immunity points out the difference between the human and several of the experimental animals. Dr. Brambell's work has been largely concerned with the rat. He shows that in this animal, fetal immunity is partially acquired before birth through the gastro-intestinal tract somewhat, but more importantly by the yolk sac, and after birth, for the first 21 days, through the gastro-intestinal tract.

The Proceedings of the Fourth Conference on Gestation are extremely enlightening to those interested in the specific fields covered. This type of conference cannot be too highly praised.

C. D. DAVIS

MEDICAL ELECTRICAL EQUIPMENT. Robert E. Molloy, Ed. New York, Philosophical Library, Inc., 1958. 312 pp. $15.00.

The criteria used in judging the usefulness of this book were its applicability to present-day American equipment, recommended standards, and practices. It was hoped that the book would furnish a source of general information about the various items of electrical equipment in use in the hospitals and doctors' offices in this country. However, the description and discussion were limited to equipment manufactured in, and standards recommended for, the United Kingdom, with virtually no mention of American standards or equipment. Moreover, in those areas which the reviewer felt competent to judge, i.e., communication, air conditioning, operating room safety, and food service equipment, the equipment recommended and standards suggested in the book have little application in this country.

For example, the nurse-patient communication equipment described is of a type long since outmoded, and no mention of voice intercommunication equipment is made. Similarly, food service equipment is antiquated and/or rudimentary by modern American standards. In the area of operating room safe practices, the recommended maximum voltages for auxiliary equipment is considerably higher than that recommended by the National Fire Protection Association. The methods and frequency of testing recommended for conductive flooring also differ from the above standards in the unsafe direction. Air conditioning is mentioned only as a word; almost the entire chapter is given over to ventilation equipment and refrigeration installation of primitive types, with no mention of the factors to be considered in comfort air conditioning. Other equipment items doubtless differ from the type currently in use here.

It should also be mentioned that since standard circuit voltages and alternating cycle frequencies differ from American practice, and a number of electrical terms differ from those in use here, a certain amount of confusion is created for the reader. A great deal of space is given over to description

of detailed maintenance procedures for items of equipment which differ considerably from American models. These procedures are not ordinarily followed by American hospital personnel because the equipment is serviced by company representatives.

On the whole, the book is not recommended for American readers.

JOHN D. THOMPSON

THE MEDICAL WORLD OF THE EIGHTEENTH CENTURY. By Lester S. King. Chicago, The University of Chicago Press, 1958. 346 pp. $5.75.

This book by Dr. King is a collection of essays which is concerned with the complexities and variables which influenced the practice of medicine in the eighteenth century, and the efforts of this discipline to progress as an art and a science. The rise of modern pathology, the development of medical ethics, methods of scientific thought and investigation, efforts toward classification of disease, and the professional struggles of physician against physician and physician against apothecary are adequately and interestingly pursued by the author.

One of the more delightful essays concerns itself with the professional career of Samuel Hahnemann, the creator of homeopathy, of whom the author writes these descriptive phrases. "Profound scholarship that lacked common sense. Penetrating intellect that could not see the obvious. Great logical acumen that ignored facts." When homeopathy's meager birth and its lack of experimental and clinical evaluation are realized, the growth and development of this discipline appears awesome. In the setting of the eighteenth century, however, homeopathy appears as a somewhat justifiable alternative to the accepted standard of medical care which advocated the administration of complex medications which contained numerous active ingredients. The patient appeared destined to receive either superfluous or insufficient therapy. Dr. King notes that Hahnemann prescribed a single pure drug for a specific malady but in a ridiculously inadequate dosage. The "shot-gun" therapy against which Hahnemann rebelled seems to be ever present.

The essay on fevers effectively describes the confusion and inadequacy of medical thinking in the eighteenth century. The possibility is then suggested that our own thinking concerning the etiology and pathogenesis of arteriosclerosis and cancer may be similarly viewed by our medical colleagues of the twenty-second century. The numerous similarities between the social, moral, economic, and intellectual problems confronting the eighteenth century medical practitioner and his twentieth century counterpart are made at once obvious and impressive to the reader of these essays.

In conclusion, I should like to borrow the author's own words inasmuch as they seem best to describe the lessons to be learned from a familiarity with the phenomenon of medicine as it existed two hundred years ago. "The practice of medicine changes constantly, just as does the art of painting or of architecture. But the soul of the artist does not significantly change, nor does the soul of the doctor."

WILLIAM L. KISSICK

A TEXTBOOK OF PSYCHOLOGY. By D. O. Hebb. Philadelphia and London, W. B. Saunders Co., 1958. 276 pp. $4.50.

The large and heterogeneous collection of books written over the past fifteen years for the first course in psychology can be fitted rather loosely into two categories—the encyclopedic and the systematic. At their worst, the "encyclopedic" text is merely sprawling and the "systematic" merely personal, but a good defense can be presented for both ways of introducing the undergraduate to a field as various as psychology. Professor Hebb has thrown his weight to the systematic side with a short and cleanly written exposition of his technique for comprehending behavioral data. Drawing on the theoretical position presented in his *Organization of Behavior,* he has brought into articulation such usually diverse topics as perception, maturation, thinking, and motivation. He manages this task of integration with wit and modesty, and without surrendering to the students' fear of physiological detail. More than this, he accepts much of the layman's language and leads the student past the pitfalls of "mind," "thought," "consciousness," and "imagery" by thoughtful analysis rather than by wholesale rejection of such terms. This respect for Everyman's point of view, together with his tendency to present empirical findings in a condensed narrative free of tables and graphs, will win friends for Professor Hebb among students, but such reasonableness may make it difficult to convince these students that they need to revise the versions of psychology they bring to the first class. This is the paradox of Professor Hebb's book. He demands a great deal of the student in his treatment of neurophysiological hypotheses (for example, 36 of 78 figures detail the nervous system and its function), but he does not tamper too much with their other preconceptions about behavior (for example, there is scarce mention of psychoanalysis, social psychology, or clinical methods, and a slim 25 pages are given to the vast field of learning). Perhaps the encyclopedists have a point—a dry recitation of empirical results does not charm or persuade, but it permits the teacher to advance his speculative integrations from a comparatively firm base. The systematic or personal textbook of psychology, in 1958, at any rate, may set undesirable boundaries on the first student's appreciation of the complex tasks psychologists face. Valuable as it is in presenting a coherent point of view, Professor Hebb's treatment of psychology can be used effectively as a *primary* textbook in the first course only by teachers already committed to his view of psychology.

WILLIAM KESSEN

TREATMENT OF THE CHILD IN EMOTIONAL CONFLICT. By Hyman S. Lippman. New York, The Blakiston Division, McGraw-Hill Book Company, Inc., 1956. 298 pp. $6.00.

The diagnostic-treatment processes utilized to help emotionally disturbed children in an out-patient psychiatric clinic form the basis for this book by Dr. Hyman Lippman, Director of the Amherst Wilder Child Guidance Clinic, St. Paul, Minnesota. The author has an extensive background in psychoanalysis and psychiatry, and a broad experience as a teacher of child psychiatry and social work. For those involved in the child guidance field,

this book presents a well-balanced description of the psychiatric team at work. The author has succeeded in describing the many considerations that are necessary for family diagnosis and treatment which are involved in psychiatric work with children. In a humble, critical manner Dr. Lippman distills his many years of clinical experience into principles and attitudes that are practical lessons for those working in a child guidance clinic.

In the first section, "Approaching Therapy," the reader will find a clear description of how the family brings the child for help, and the various avenues available to the psychiatric team to involve the parents in the child's therapy and to introduce the child to his need for treatment. The psychiatric team consists of the psychiatrist, social worker, and clinical psychologist. Dr. Lippman gives deserved credit to the ordinary devoted mother. However, there are several instances where he uses the concept of the rejecting mother, at the same time as he agrees with Anna Freud that this concept is unrealistic and that it can hinder a fruitful treatment plan.

In the next four sections the reader is provided with a diagnostic classification that is based more on the description of the patient's problems and the indicated treatment than on etiology. However, the careful blending of theoretical and clinical considerations are used by the author to demonstrate that multiple factors have to be considered in etiology.

The author expresses the idea that emotional tension and conflict produce pathological states. He would have been clearer if he had pointed out that tension and conflict are as necessary for health as for illness. How the individual copes with these states determines whether the outcome is adaptive or maladaptive.

Among the most valuable and richly rewarding sections of this book are those which describe the social worker's role with disorganized families, the considerations involved in recommending against treatment for the child, the assessment of the physical, psychological, and social factors in a particular child and his family, and the sections in which the role of foster parents and residential treatment centers are described. The many interesting clinical vignettes are illustrative of Dr. Lippman's approach, and they reflect the rich clinical background of this significant book.

ALBERT J. SOLNIT

FOUNDATIONS OF EMBRYOLOGY. By Bradley M. Patten. New York, McGraw-Hill Book Co., Inc., 1958. xvi, 578 pp. $10.50.

The author of this book is well known for his numerous contributions in the field of embryology. Many undergraduate students in zoology have studied his textbooks on *Early Embryology of the Chick* and *Embryology of the Pig* and a large number of medical students have read with great profit and pleasure his textbook on *Human Embryology.*

Professor Patten's latest book, *Foundations of Embryology,* is designed to tell the story of vertebrate development as simply and clearly as possible to students beginning a course in embryology. He has drawn heavily upon the material published in his previous books.

Although in the fields of comparative embryology and experimental embryology lie some of the most valuable areas for viewing a picture of development, Patten has chosen to enter these fields only lightly. He believes that for a beginning, fundamental course in embryology it is far more important to give a smooth-running account of a continuously changing picture so that the student keeps familiar with the trends of events in development without becoming lost on the way by the introduction of comparative and experimental data. Many of these data he would leave for a more advanced course.

Among teachers of embryology there will no doubt be differences of opinion as to how this can be handled. However, it must be said that Professor Patten has distributed much information concerning the comparative and experimental data at appropriate moments in his story. He has very successfully presented a smoothly written account of a continuously changing picture, well illustrated by 962 drawings and photographs.

The twenty-two chapters are divided into three parts. Part One gives the story of the early developmental processes common to all vertebrates, from the formation of eggs and sperm related to the sex cycle, through fertilization, cleavage, and the formation of the primitive germ layers. It ends with a good clear account of the primitive streak and a map of the presumptive organ-forming areas around it. The illustrative material for this section has been drawn from amphibians, birds, and mammals.

Part Two is devoted to the establishment of the body and the laying down of the organ systems. The picture here is confined entirely to the chick to show the trend of embryological events as a continuous story without interruptions to explain differences in various animals. For this the chick is ideal.

Part Three deals with the later stages in the development of the organ systems, as illustrated in the embryos of pig and man. To connect the story with that of the chick, it begins with a brief account of the mammalian primitive streak and then unfolds the external and internal features of the basic body plan. The implantation of the human blastodermic vesicle and the development of the foetal membranes related to the maternal uterine tissues are well described and illustrated. As the internal organs become more complicated, the course of events is followed quite clearly, by examining the development of one organ system at a time. The story ends with the completion of the circulatory system and the changes in it following birth.

There are twenty-two pages of references for collateral reading. Most of them are divided into sections, each of which refers to chapters in the text. There is also a good index.

This is a very good textbook for an elementary course in embryology. Some of the lecture material associated with such a course could provide the student with many exciting illustrations drawn from the rich literature of experimental embryology.

L. S. STONE

STANDARD METHODS OF CLINICAL CHEMISTRY, VOL. II. The American Association of Clinical Chemists. David Seligson, Editor-in-chief. New York, Academic Press Inc., 1958. 217 pp. $5.50.

This book belongs on the reference shelves of clinical chemical laboratories, but would not be as valuable to strictly research laboratories. It is worth its price to obtain certain invaluable chapters: Sodium and Potassium by Flame Photometry by Hald, an authority on this subject, Nitrogen by the Kjeldahl Method by Archibald, an experienced investigator, and Free and Conjugated 17-Hydroxycorticosteroids in Plasma by the expert chemist Bongiovanni.

Twenty-one chemical anlyses are described clearly and precisely. These techniques cover a wide range of subjects, some of which have already been mentioned. Other chapters extend from chlorides to porphyrins in urine, alkaline and acid phosphatase, pancreatic lipase, total fatty acids in stool, gamma globulin in serum. In specific diagnostic problems some of the 21 techniques may be needed suddenly. For such emergencies this book has distinct merit since definite directions can be followed without reference to numerous journals for improvements published at odd intervals. Except for two chapters, the methods, as described in detail, have been submitted to referees in independent laboratories who verified their accuracy by following completely specific directions for reagents and techniques. This checking by referees increases the step-by-step lucidity of each manipulation. The tests, as described, have increased diagnostic value because most of the chapters contain comments on normal values or interpretation of results.

On the other hand, the investigative clinical chemist must choose what technique best suits his conditions and the brevity of the book limits each chapter to description of a single technique. Bibliographies with inclusion of titles of articles are limited in most instances to between 4 and 20 references. Selection of one technique of course opens the door to criticisms of which the following examples are cited. The chapter on Calcium (Compleximetric) describes a method unknown to directors of some hospital chemical laboratories. Zilversmit, a lipid expert, describes trichloroacetic acid precipitation of phosphatides from serum without inclusion of remarks about the completeness of precipitation of phosphatides from grossly lactescent sera. Possible modifications of isolation of phosphatides by lipid solvents are described without mention of the comparative accuracy of these modifications for lactescent sera. The chapter on Protein-Bound Iodine recommends omission of any medication containing inorganic iodine for 24 to 48 hours prior to the test, but does not refer to the prolonged spurious elevation of PBI from ingestion of inorganic iodine observed by Danowski and others.

The book is in clear type and excellently edited with uniformity. To describe methods for 21 clinical tests in an arduous task, especially in a field in which instrumental improvements subject techniques to continual change. However, this volume belongs with technical manuals rather than with outstanding clinical classics.

EVELYN B. MAN

ADDITIONAL BOOKS RECEIVED

Instinctive Behavior; the Development of a Modern Concept. Translated and edited by Claire H. Schiller. New York, International Universities Press, Inc., 1957. 328 pp. $7.50.

Aggression. By John Paul Scott. Chicago, Illinois, University of Chicago Press, 1958. 148 pp. $3.75.

The Problems of World Population. By Sir Charles Darwin. Cambridge, The University Press, 1958. 40 pp. $0.75.

The Health of Israel. By Moses Einhorn. Reprinted from Vol. II, 1957 of the Hebrew Medical Journal.

Human Potentialities. By Gardner Murphy. New York, Basic Books, Inc., 1958. 340 pp. $6.00.

A Search for Man's Sanity. Selected Letters of Trigant Burrow. Prepared by the Editorial Committee of the Lifwynn Foundation. New York, Oxford University Press, 1958. 615 pp. $8.75.

The Negro Personality. . . . By Bertram P. Karon. New York, Springer Publishing Co., 1958. 184 pp. $4.50.

Labor Union Monopoly, a Clear and Present Danger. By Donald R. Richberg. Unabridged special ed. Chicago, Henry Regnery Co., 1957. 177 pp. $3.50.

Laboratory Instruction in Microbiology. By Louis P. Gebhardt and Dean A. Anderson. St. Louis, C. V. Mosby Co., 1958. 261 pp. $3.75.

A Periodic Table for Fundamental Particles. By John J. Grebe. Annals of the New York Academy of Sciences, vol. 76, art. 1, pp. 1-16. New York, 1958.

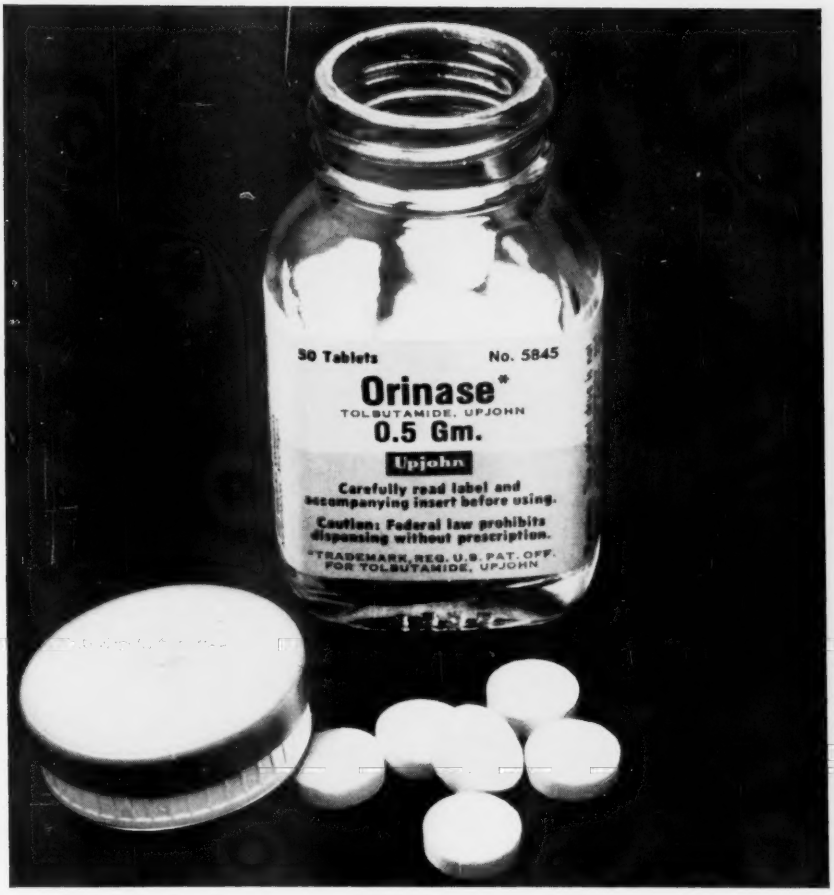
The influence of Sulfhydryl Groups and their Inhibitors on the Distribution of Radiocobalt in the Organs and Intracellular Organelles of the Mouse. By Laurence S. Maynard. Annals of the New York Academy of Sciences, vol. 72, art. 6, pp. 227-238. New York, 1958.

Basic Biodynamics. By Edward J. Kempf. Annals of the New York Academy of Sciences, vol. 73, art. 4, pp. 869-910. New York, 1958.

Contributions of the Physical Biological and Psychological Sciences in Human Disability. By Renato Contini [and others]. Annals of the New York Academy of Sciences, vol. 74, art. 1, pp. 1-160. New York, 1958.

British Medical Bulletin, vol. 14, no. 2. *Causation of Cancer.* London, Medical Dept., The British Council, 1958. 195 pp. $4.00.

British Medical Bulletin, vol. 14, no. 3. *Metabolism of Lipids.* London, Medical Dept., The British Council, 1958. 197-278 pp. $3.25.



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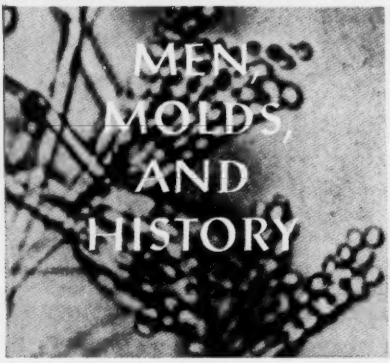

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